STUDIES OF METHODS OF ESTIMATING BODY COMPOSITION IN THE LIVING PIG

By

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DECLARATION

This thesis was composed by the writer and is a record of the work carried out by him on an original line of research. All sources of information are shown in the text and listed in the Bibliography and all help given by others is indicated in the acknowledgements.

None of the work recorded here has been presented in any previous application for a degree.

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C O N T E N T S

I have noted the following errors in the text:

Page 101, Table 7.8 For the prediction of percent lipid in the empty body, the regression equation should read: $y = 87.06 - 1.00\ D_2O/L.wt.$

Page 102, 6th line from the bottom, should read '... D_2O crossed the gut wall and ...'

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GENERAL SUMMARY

1. (a) The objective of the investigation was to compare the relative accuracy of several indirect methods used singly and in combination, for predicting body composition in the 90-kg pig. There were two phases in the experimental part of the study.

(b) The first phase was concerned with the development of the potassium 42 (^{42}K) and deuterium oxide (D_2O) dilution techniques and their application to bacon pigs. In addition, three other dilution techniques, Evans Blue, sodium thiocyanate and urea were applied to two sows for the specific purpose of determining their suitability for inclusion into the second part of the study.

(c) In the second phase of the study, several indirect techniques were applied simultaneously to 24 bacon pigs. These were the dilution of administered ^{42}K, of D_2O and of Evans Blue, various external measurements, ultrasonic measurements of backfat thickness, visual appraisal, and the measurement of the feed conversion ratio adjusted for the estimated food requirements (C.F.C.R.). Measurements of the specific gravity of the chilled half-carcass and backfat thickness at several points were made after the pigs were slaughtered.

2. (a) In the first phase, ^{42}K injected into 17 bacon pigs was found to equilibrate with about 97% of the body potassium in 10 to 12 hours. The loss of the label from the body in 22 to 28 hours was on average 4.9% of the total label injected, the main loss being in the urine. Exchangeable potassium (K_{eu}) determined from the urine specific activity was highly correlated with ($r = 0.865$) chemically-determined body potassium. The relationships between K_{eu} and the fat-free weight ($r = 0.93$) and percentage body lipid ($r = 0.92$) were extremely close

and the Residual Standard Deviations (R.S.D.) of the regressions were ± 1.80 kg of fat-free material, and ± 2.5% body lipid.

(b) In ten of the pigs used in the ^{42}K investigations, body water was estimated by D_2O dilution. D_2O equilibrated in the body in 2 to 3 hours. The amount of D_2O found in the faeces and gut contents amounted to 3% to 4% of the total label injected. Only small quantities of D_2O were found in the urine, but this was found difficult to analyse. A comparison of the D_2O space with the empty body water and total body water showed that D_2O gave fairly accurate estimates of the water in the empty body ($r = 0.94$ R.S.D. = ± 1.06 kg) and in the whole body ($r = 0.89$ R.S.D. = ± 1.59 kg), despite the large discrepancies which were often found between D_2O space, empty body water and total body water.

(c) Body water was estimated by urea dilution in two sows. The variation in the slope of the dilution curve indicated that there was either a variable endogenous production of urea or that the exogenous urea was metabolised at different rates after its injection. Over a period of three days the urea space in one sow was observed to be extremely variable (Mean = 83.38 litres S.D. = ± 8.740 litres) and considerably exceeded the D_2O space (Mean = 73.98 litres S.D. = ± 1.008 litres). The urea dilution method was not considered sufficiently reliable for estimating body water.

(d) Extracellular water space was estimated in two sows by sodium thiocyanate dilution. The tracer equilibrated in the body in about $1\frac{1}{2}$ to 2 hours, but when repeat determinations of the extracellular space were made in one of the sows, the values obtained were found to be extremely variable (Mean = 72.5 litres S.D. = ± 7.36 litres) and were similar to the D_2O space (Mean = 73.98 litres S.D. = ± 1.008 litres).

The method was considered too unreliable for inclusion in the second part of the investigation.

(e) Blood volume was estimated in one sow by Evans Blue dilution in combination with haematocrit measurements. Determinations of blood volume made over a period of three days were found to be very similar, the mean value being 10.53 litres and the S.D. $\pm$ 0.436 litres. The method was considered to be reliable for estimating blood volume in the pig and was included in the second part of the study.

3. (a) In the second phase of the study in which several indirect methods were applied simultaneously to each of 24 animals, regression equations were computed between each of the predictors and the chemical and dissectible components of the body, first in isolation, and then in combination with other predictors.

(b) For the dilution techniques ^{42}K, D_2O and Evans Blue, the correlations between the exchangeable potassium, total body water, estimated blood volume, and the fat-free weight were 0.976, 0.948 and 0.819, respectively, and the R.S.D's of the regression equations were 1.347 kg, 1.876 kg and 3.385 kg, respectively. For the prediction of body lipid, the inclusion of liveweight as an independent variable into the equations, significantly improved the accuracy of estimation. Generally, it was found that the dissectible components of the body were not predicted with the same accuracy as were the chemical components.

(c) The results obtained for the ultrasonic technique showed that measurements recorded at the shoulder, midback and loin were more highly correlated with body lipid and dissectible fat than were those recorded on the latero-dorsal surface at $4\frac{1}{2}$ cms (C) and 8 cms (K) from the mid-line. Conversely, on the chilled carcass it was found that the C and K measurements were more highly correlated with the body lipid

and dissectible fat than were the fat depths recorded at the shoulder, midback, or loin.

(d) Measurements of the external dimensions of the body were not found to be highly correlated with any of the body components. The highest correlations were those between neck circumference and body lipid ($r = 0.700$) and dissectible fat ($r = 0.700$). The length of the fore-arm in combination with liveweight was fairly closely correlated with the fat-free weight ($R = 0.719$), the R.S.D. of the prediction equation being 4.144 kg.

(e) Visual appraisal of the pigs by a panel of five judges was found to be relatively accurate in predicting the weights of dissectible fat and body lipid. The mean panel score was closely correlated with body lipid ($r = 0.844$) and dissectible fat ($r = 0.810$), the R.S.D's of the prediction equations being 2.551 kg and 2.003 kg, respectively.

(f) Feed conversion ratio was not significantly correlated with any of the body components, but when an adjustment was made for the estimated energy requirements of maintenance, relatively high correlations were obtained with several body components. The correlations with body lipid and fat-free weight were 0.953 and 0.943, respectively, the R.S.D's of the prediction equations being 1.473 kg and 2.007 kg, respectively.

(g) Specific gravity measurements made on the chilled half-carcass were found to be highly associated with the composition of the empty body. The correlations with body lipid and fat-free weight were 0.965 and 0.977, respectively, the R.S.D's of both prediction equations being 1.286 kg. Analysis of the data showed that the relative proportions of lean and fat in the carcass were highly correlated with carcass specific gravity ($r = 0.903$) but there was a non-significant correlation between lean/bone ratio and carcass specific gravity ($r = 0.224$).

4. (a) From the data obtained in the second phase of the investigation, combinations of several predictors were synthesised and extremely accurate estimates of the major components of the body were obtained. By combining together liveweight, potassium 42 space, C.F.C.R. and ultrasonic loin fat depth, the weight of body lipid could be predicted to $\pm$ 0.850 kg, the multiple correlation being 0.987. By combining liveweight, potassium 42 space, and C.F.C.R., the fat-free weight could be predicted to $\pm$ 0.889 kg, the multiple correlation being 0.990.

(b) Examples of four situations related to animal production were considered, in which a determination of body composition was required. In each situation it was found that combinations of certain predictors gave more accuracy in the estimates of body lipid, fat-free weight, dissectible fat and dissectible lean, than any of the predictors used in isolation.

GENERAL INTRODUCTION

The structure and composition of the bodies of animals and man has been, throughout history, a source of fascination. The condition of people who are either obese or excessively thin has always provided an interesting talking-point, and the desire to possess a pleasing body shape is becoming an obsessive preoccupation of many societies.

In agriculture the form of domesticated meat animals has also stimulated man's curiosity. It was not, however, until quite recent times that it was realised that their shape and composition could be affected by the farmer. Early attempts at manipulating form, resulted in such oddities as the greatly admired "Durham ox". The emergence of industrial centres of population in the middle of the 19th century brought about a revolution in meat production and marketing. The times of the almost ritualistic event of the slaughter of the cottager's pig or of his sheep or cattle, were quickly passing and the meat industry was transformed into a complex operation. Animals were dispatched to large-scale abbatoirs employing factory techniques for slaughter, processing and marketing.

The Danes were among the first to recognise that the consumer public had strong preferences for the characteristics of the meat they consumed. They defined carcass quality for the pig in objective terms. The New Zealanders were the leaders of a similar development in sheep production and the impact of these developments has been felt by all the major meat producing industries of the western world.

The importance of carcass composition, and its relationship to quality is now widely recognised, but even today, the bulk of the trade in meat animals between farmer and butcher is based on the subjective appraisal of the animal on the hoof, and only limited attempts have been made to

establish the relationships between measurements taken on the live animal with the composition of the resulting carcass.

There is an obvious requirement for the assessment of *in vivo* body composition in many agricultural situations. In breeding programmes a knowledge of the composition of multiplying stock at the end of performance tests is required for large-scale centralised schemes and at individual farm level. In the wider context of applied biological research there is an urgent requirement in many areas for the measurement of sequential changes in body composition. In this context there is an interface between agricultural research and biomedical research and the exchange of indirect techniques from one discipline to the other is an important factor in the progress of each.

The quantification of the constituents of the live animal has been investigated by many methods. The interest in this field is reflected in the number of symposia and meetings held in the last twenty-five years reporting the newest techniques. Despite the effort which has gone into the development of these methods, there has been little attempt to fit them into practical situations, or even to indicate their value as a research tool. The potential merit of some methods may have been overlooked because of the limited context in which they were first developed.

A serious limitation of much of this work is that the indirect methods were considered in isolation from each other. There is a need for work which will form the basis of a strategy for assessing the value of techniques used individually or in combination in particular situations.

The purpose of the work described in this thesis is to assess the merits of different methods of estimating body composition in the live pig. The pig is admirably suited for this work because it is a species in which further advances in breeding may depend on the improved ability to

estimate *in vivo* composition. Problems in *in vivo* body composition determinations in the pig are similar in many ways to those in humans, and the exchange of information is therefore of unusual potential value. Among the rest of the pig's assets for work of this type, is the considerable experience which has already been gained in techniques of body composition analysis and measurement.

The thesis is presented in three parts. In the first, the concepts which have become established in this area of agricultural research are introduced. Their value and relevance to body composition problems is discussed. The purpose of the review of the indirect techniques which follows is to allow the selection of a representative group of promising techniques to be made. The errors which can arise in the application of these techniques are also discussed.

The objective of the second part is the formulation of the experimental programme. A historical review of the development of concepts and techniques which have been proposed for the measurement of *in vivo* body composition, is given. Their relevance to the current demands of the agricultural industry is discussed. Some of the more promising techniques are chosen for the present investigation, and the development of a new method is also described. The application of several of these methods to the pig is attempted, both in isolation and in combination with each other.

The aim of the third and final part is to show the relative value of each of the indirect methods applied. The results of each method are presented first of all individually, and then combinations of predictors are synthesised to suit different agricultural situations. The success of the whole project is then discussed in terms of its contribution to the agricultural industry.

Part 1

REVIEW OF THE LITERATURE ON BODY COMPOSITION

CHAPTER 1

CONCEPTS IN BODY COMPOSITION

Introduction

Our understanding of body composition is largely dependent on the knowledge gained from experimentation involving the slaughter and analysis of animal bodies. The experiments of Lawes and Gilbert at Rothamsted in 1859 quantified for the first time the proportions of the constituents of the body in several domesticated species. Since that time many detailed analytical studies have been made and the subject of body composition now has a substantial literature, a wide range of concepts and a terminology of its own.

The purpose of this chapter is to examine the genesis of those concepts which have proved most useful in this field and show how they integrate with that most central of biological concepts, the principle of physiological homeostasis.

Physiological homeostasis

This concept was first expressed by Bernard (1878) when he suggested that the reactions of the organism to changing conditions were directed at maintaining constant its 'milieu interieur'. Subsequently Cannon (1929) developed the concept giving it the present name of physiological homeostasis.

The principle is a simple one, in that the survival of the animal depends on its ability to provide a constant environment at the cellular level where the essential life processes are carried out. This generalisation has been repeatedly found by experiment to hold true in terms of

chemical, physical and physiological parameters. For example, the chemical composition of fat-free muscle, the major tissue in all mammalian species from mature animals is surprisingly constant (Lawrie, 1966).

The operation of this principle greatly simplifies the concepts which are needed in describing body composition in farm animals. Relationships between body components either chemically or physically defined are not random, but are closely governed by control mechanisms ensuring that physiological homeostasis is maintained. An understanding of the underlying physiology therefore allows the selection of relationships which potentially may have a useful degree of correlation.

Chemical concepts

The success of the simplified concepts of the animal can be largely attributed to the universal relevance of the homeostatic principle. The division of the animal body into fat and fat-free material relies on the assumption that the composition of the latter is relatively constant and is determined by the animal's fundamental physiology.

Murray (1922) advanced the concept of the 'fat-free body' after analysing some of the early work on body composition (Lawes and Gilbert, 1859). In his investigations Murray pointed out that the chemical composition of the carcasses of farm animals was fixed, once the proportion of fat was known, because the composition of the non-fatty material was the same in all animals and was not affected by fatness and varied only slightly with age.

In a number of species Moulton (1923) demonstrated that the electrolyte, water and protein contents of mammals decreased rapidly from conception to birth and then decreased less rapidly until a relatively constant concentration was reached. The animals were then considered to be chemically mature at this point. For pigs, chemical maturity was reached

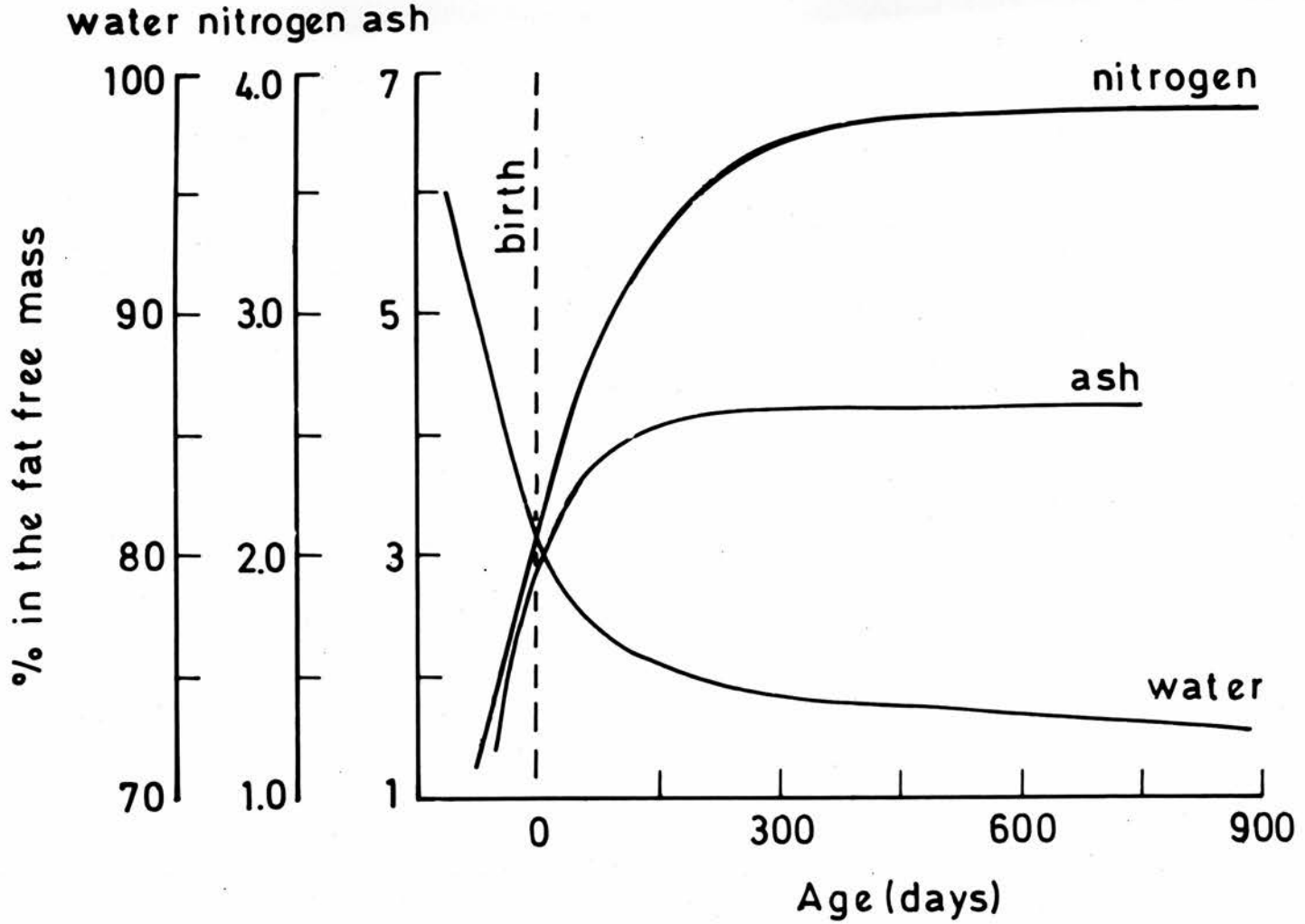


Fig.1.1 The Effect of Age on the Composition of Pigs
(after Moulton 1923)

between 150 and 300 days (Fig. 1.1). Later Spray and Widdowson (1951) showed that there was a considerable variation in the age at which the concentration of the principal constituents in the fat-free mass became stabilised. More recent studies have also quantified the proportions of the constituents in the fat-free mass for several species, and the proportions of water in the fat-free mass determined in a number of species are shown in Table 1.1. It is clear that between and within species the water content of the fat-free mass is remarkably constant.

Table 1.1

The water content of the fat-free mass in several animal species

Species	Water content of the fat-free mass			Source
Guinea Pig	72.4	74.2		Pace and Rathbun (1945); Hatai (1917)
Rat	71.8	74.4	72.6	Pace and Rathbun (1945); Ashworth and Cowgill (1938); Light, Smith, Smith and Anderson (1934)
Rabbit	73.5	76.3	72.6	Pace and Rathbun (1945); Harrison, Darrow and Yannet (1936); Panaretto (1963)
Man	73.2	77.5	69.4	Pace and Rathbun (1945); Mitchell, Hamilton, Steggarda and Bean (1945); Forbes, Cooper and Mitchell (1953)
Cattle	72.6			Kraybill, Bitter and Hankins (1952)
Sheep	74.0	76.6	77.6	Reid, Bensadoun, Paladines, Van Niekerk (1963)
Pig	74.4	75.3	74.6	Kraybill, Goode, Robertson and Sloane (1953); Clawson, Sheffy and Reid (1955); Gnaedinger, Pearson, Reineke and Hix (1963)

The relatively constant proportions of many constituents of the fat-free mass reported in several investigations have given rise to several concepts which are commonly referred to in compositional studies. The total body water space, various electrolyte spaces (e.g. potassium space, chloride space) and body protein and lipid are examples of these concepts which are used to describe body composition of many species in both normal and abnormal physiological states.

Despite the convincing evidence on the constancy of the fat-free mass, several workers (Keys and Brožek, 1953; Gnaedinger, Pearson, Reineke and Hix, 1963) have indicated that the composition of the fat-free mass is influenced by the extent of body fatness. On analysis of the data of Pace and Rathbun (1945) Keys and Brožek obtained a correlation coefficient of -0.45 between the proportion of body fat and the water content of the fat-free mass. Gnaedinger et al. (1963) obtained a correlation coefficient of -0.58 between these two parameters in the pig. Indeed there is a great deal of supporting literature (Spray and Widdowson, 1951; Reid, Bensadoun, Bull, Burton, Gleeson, Han, Joo, Johnson, McManus, Paladines, Stroud, Tyrrell, Van Niekerk and Wellington, 1968), which shows that with increasing age and weight there are changes in the composition of the fat-free mass; but there is no evidence to suggest that the main source of variability is the quantity of body fat. It is most likely that in the animals studied by Pace and Rathbun (1945) and Gnaedinger et al. (1963) the water content of the fat-free mass was changing with increasing age and weight, irrespective of the amount of fat present. Supporting evidence comes from the data obtained on small laboratory animals. Bailey, Kitts and Wood (1960) found no difference in the mean percentage of water in the fat-free mass between genetically obese mice and their lean siblings.

The changing nature of the fat-free mass during growth as reported

by Spray and Widdowson (1951) and Reid et al. (1968) has been explained by several workers as changes in the proportions of the components of the fat-free mass. These workers considered the fat-free mass not as a single uniform entity, but as a multicomponent entity. The fat-free mass in the models proposed by Von Döbeln (1962) and Anderson (1963) consisted of muscle and muscle-free lean which helped to explain the variation in water/potassium ratios with increasing age.

Although these increasingly, arbitrary subdivisions of the body can theoretically explain the variability in the proportions of the chemical constituents, in practical studies it is often more useful to consider the simple concepts such as the fat-free mass/lipid model of body composition.

Physical concepts

The physical concepts of body composition have assumed importance mainly as the result of the findings of the classical experiments on growth and development in several mammalian species. In general these experiments have demonstrated that the differential growth rates of the components of the body are responsible for the changes in body composition during the postnatal period (Hammond, 1932; Pálsson and Vergés, 1952; McMeekan, 1940; Elsley, McDonald and Fowler, 1964).

Although the fixed nature of the relationships between the body components during growth have been clearly demonstrated (Huxley, 1932), later studies on pigs (McMeekan, 1940) and lambs (Pálsson and Vergés, 1952) showed that allometric relationships did not hold under certain conditions. The relationships between the weights of organs to the weight of the whole body were different on the different nutritional regimes. However, in more recent experiments with goats (Wilson, 1960) and lambs and pigs (Elsley, McDonald and Fowler, 1964) it has been shown that the relationships between the components of lean tissue can be described by the allometric

equation of Huxley (1932) when the analysis is carried out on a fat-free basis.

The concept of fat-free tissue is clearly important in assessing the response of the animal to its environment. Clearly, related concepts such as dissectible fat, muscle and bone are also useful in body composition studies because of their relationships with the fat-free material of the body.

Physiological concepts

There are several concepts used in body composition work which have assumed importance because of the requirement for descriptions which would be common to many species, and to which certain physiological factors could be referred. For example, Behnke (1943) put forward the concept of the lean body mass (LBM) which was composed of water, protein, minerals and a small amount of "essential lipid". In 1959 Ljunggren proposed the concept of "non-obesity tissue" which consisted of muscle, muscle-free lean and bone. Although these components cannot be accurately defined in physical or chemical terms they have been used extensively in many studies as reference bodies.

Other reference bodies which are commonly used in body composition studies are the active protoplasmic mass, extracellular water, intracellular water and blood volume.

Summary

The principle of homeostasis proposed by Bernard (1878) has been shown to be an important concept in many studies of growth and development in farm animals. The far-sightedness of agricultural researchers at the turn of the century in their examinations of the chemical constituents of the animal body on a fat-free basis, and the more recent contributions

have demonstrated that the fat-free material of several mammalian species has a very constant composition. The concept of the chemically stable fat-free mass is obviously important in body composition studies.

A number of other workers have shown that slight changes do occur in the constituents of the fat-free material and alternative concepts have been proposed to help explain and quantify these changes, although they have had little application in more practical studies.

Three different types of concept used in body composition studies have been introduced. These are the chemical, physical and the physiological concepts. All of these concepts are important in _in vivo_ studies, the exact emphasis depending on the purpose of the study, and the techniques which are available.

CHAPTER 2

TECHNIQUES FOR MEASURING BODY COMPOSITION IN THE LIVE ANIMAL

Introduction

The techniques available for measuring body composition can be conveniently classified into two groups, direct and indirect. The indirect methods can be further classified into those which rely on measurements taken from only part of a body component, and those which rely on measurements of a whole component.

Direct methods are not applicable to man, for obvious reasons. Data on the chemical composition of the human body are limited, and therefore indirect methods can only be compared with each other (Remenchik, Miller and Kessler, 1968). For small laboratory animals and domesticated meat animals, there have been many experiments concerned with the direct measurement of body composition, in which the growth of the physical and chemical components of the body have been monitored during the animal's development (Hörnicke, 1959; Bailey, Kitts and Wood, 1960; Reid et al., 1968; Callow, 1947; Tulloh, 1963). The information obtained from these experiments is useful for assessing the response of the animal to its environment, and is also useful to those with commercial interests, because a measurement of the meat value of the total carcass can be made from the physical composition of various representative cuts (Lush, 1926; Hankins and Howe, 1946; McMeekan, 1941).

Direct methods of estimating body composition in animals have the disadvantage in that only one assessment can be made for each animal, usually involving laborious procedures in which the errors in sampling and

analysis are often large (Morris and Moir, 1964; Hill and O'Carroll, 1962).

In this chapter an attempt will be made to review some of the techniques which have been used for assessing in vivo body composition in animals. The methods which were considered the most suitable candidates for inclusion in the present investigation are discussed in greater detail in Chapter 6.

Non-dilution techniques

(i) External measurements. The external dimensions of an animal are generally considered to be indicative of the amount of bone, simply because they depend largely on the size of the animal, which in turn is a function of skeletal size (McMeekan, 1941). The measurements of external dimensions for predicting skeletal weight are not considered to be very reliable because (a) they are affected to some extent by the flesh cover of the animal (b) there are difficulties in defining the positions or points between which they should be taken (c) there is often movement of the animal and a change in posture.

McMeekan (1941) obtained some high correlations between external dimensions and skeletal weight and the dissectible muscle in the carcass. The correlation between the length of the fore trotters and skeletal weight was 0.7666. This relationship was improved ($r = 0.8400$) when the length of the fore-arm was included. Depth of chest was found to be non-significantly correlated ($r = 0.0418$) with skeletal weight.

The length of the fore-trotter was found to have the highest correlation with the dissectible muscle in the carcass ($r = 0.8036$) but other measurements such as circumference of the base of the tail and circumference of forearm were not highly correlated with carcass muscle.

The weight of fat in the carcass was not related to any external measurement, but high correlations were found with several backfat measurements. The fat depth at C and the loin fat depth had correlations

of 0.9663 and 0.9312, respectively, with the weight of carcass fat.

(ii) The backfat probe. Hazel and Kline (1952) were the first workers to measure the thickness of subcutaneous fat of the living pig by means of a probing device. A metal ruler is pushed through an incision in the skin and fat until it reaches the underlying muscle bundles. The procedure causes little discomfort to the pig. The measurement at the location, one and half inches off the midline immediately behind the shoulder, was found to be the most accurate single predictor of carcass fatness ($r = 0.79$). In a further study in 1953, using bacon pigs, Hazel and Kline found that probe measurements taken behind the shoulder and over the loin were closely related to the percentage lean and fat cuts in the carcasses. De Pape and Whatley (1956) also found that backfat depth measured by the probe in six regions was highly correlated with the percent lean cuts ($r = -0.57$) as were the direct carcass measurements ($r = -0.66$). The mean value from only two probe measurements, however, gave little indication ($r = -0.07$) of the percentage carcass lean.

(iii) Electrical probe ('Lean-meter') This technique for measuring backfat in the live animal relies on the finding (Banfield and Callow, 1935) that the electrical resistances of muscle and fat are different. The principle of operation is similar to the backfat probe, except that the change from fat to muscle is detected by a change in electrical resistance. In 1955, Andrews and Whaley devised the "Lean-meter" for use with live pigs, and in 1956 Berg and Bowland showed there was a close agreement ($r = 0.80$) between the mean of three carcass backfat measurements and the mean of the three "Lean-meter" measurements on 105 pigs. In a comparison of the Lean-meter and the live-probe in 99 pigs of both sexes, Pearson, Price, Hoefer, Bratzler and Magee (1957) indicated that there was little difference between the two methods in their relationship with actual backfat thickness ($r = 0.71$ and 0.70, respectively), but

suggested that the live-probe was a more reliable measure for estimating carcass leanness.

(iv) Ultrasonic measurements. The principle of ultrasonic reflectance is that when sound waves pass through the boundary between tissues which have a different density, an echo is produced. Ultrasonic waves refer to sound waves vibrating at a frequency above the audible range of the human ear. The generation of ultrasonic waves is normally accomplished by passing a high-frequency electrical pulse through a piezo-electric crystal which in turn expands and contracts at the same pulse frequency, generating sound waves. The time interval between the pulse and the echo is measured electronically and portrayed on an oscilloscope. The distance of the echo source can be calculated from the time interval and the speed of sound.

The use of ultrasonic reflectance as a method of carcass appraisal in meat animals has followed its diagnostic use in medical research (Wild, 1950), and early work with cattle and pigs was encouraging. Price, Pfost, Pearson and Hall (1958) found close relationships between ultrasonic readings taken at various points on the back and the carcass fatness, and Lauprecht, Scheper and Schroeder (1957) and Kliesch, Newhaus, Silber and Kostzewske (1957) concluded that ultrasonic measurements of backfat thickness in pigs were sufficiently accurate for practical application.

Analysis of the data from the literature indicates a wide variation in the relationships between ultrasonic fat and lean measurements and the respective carcass measurements. There are several sources of error which are difficult to control, and only a rigidly standardised procedure in recording the measurements can reduce these errors. Sources of error can be attributed to animal, machine and operator factors (East, Taylor, Miller and Widdowson, 1959). However, several experiments have demonstrated the accuracy of the ultrasonic technique in measuring various carcass dimensions

in the living animal which are known to be highly correlated to body composition.

Dilution techniques

The basic procedure involved in dilution techniques consists of administering, orally or by intravenous injection, a known amount of tracer which will become distributed uniformly throughout a compartment in the animal body. The concentration of the tracer at equilibrium is measured in a fluid sample such as blood or urine. The volume (V) of the fluid compartment is then given by:-

$$V = \frac{\text{Amount of tracer administered (g)}}{\text{Concentration of tracer at equilibrium (g/litre)}}$$

For the technique to be reliable the tracer should fulfill the following conditions. It should

(1) Rapidly and uniformly distribute itself throughout the fluid compartment.

(2) Not penetrate into other body compartments.

(3) Not be adsorbed or become combined with other constituents of the body.

(4) Not be rapidly metabolised by the animal.

(5) Not be toxic.

(6) Not be eliminated from the body before equilibrium has taken place. (If the solute leaves the compartment in significant amounts during the period between administration and sampling, the rate of excretion must be capable of measurement.)

The dilution spaces which will be considered are total body water, plasma and blood volume, extracellular fluid volume, exchangeable potassium and body lipid.

(i) Urea. Urea has many attributes which make it ideal for the measurement of total body water (TBW). It is non-toxic, penetrates all membranes easily and is easy to administer and to determine (Painter,

1940). In applying the method it is assumed that: (a) the rate of endogenous urea formation is constant throughout the experimental period, (b) that diuresis induced by ingestion of large amounts of urea does not significantly affect the results. These assumptions have been criticised by several workers who have found both a variable endogenous production of urea and a possible diuretic effect (Pace, Kline, Schachman and Harfenist, 1947; Levitt and Guadino, 1950; Brodie, 1951).

After infusion of urea into the body there is an initial mixing period which is characterised by a rapid decline in urea blood concentration, which is followed by a less rapid fall. If it is assumed that the rate of excretion is the same during the mixing period as it is in the latter part of the equilibration period, the initial concentration (which would be obtained if complete distribution were instantaneous, or if none of the substance left the body) can be determined by extrapolating the straight portion of the curve back to the injection time (T_o) (Painter, 1940; San Pietro and Rittenberg, 1953; Bradbury, 1961). The rate of disappearance of the exogenous urea has been found to be independent of the dose administered (Painter, 1940), and for all calculations of blood urea concentration, corrections must be made for the endogenous urea concentration.

Despite its many advantages urea is not widely used as an <u>in vivo</u> technique for measuring TBW. There are four possible reasons for this: (a) the variable endogenous production of the solute, (b) its possible diuretic effect, (c) its rapid excretion compared with the rate of distribution, (d) unequal distribution throughout the fluid space. The repeatability of the method has never been tested as it is impossible in such studies to distinguish between the changes in body water and errors inherent in the method.

(ii) Antipyrene. Antipyrene (AP) was first investigated by Soberman, Brodie, Levy, Axelrod, Hollander and Steele (1949) for the determination of T.B.W. in dogs and man. On dissection of the dogs, it was found that the ratio of tissue AP/plasma AP was approximately unity (1.01) for the whole body, and that the AP concentration in the tissues was determined chiefly by their water content. Other advantages of AP were found to be its non-toxicity, its capability of uniform and rapid distribution throughout the body tissues, its ease of chemical determination and its low excretion rate in man, which was found to be constant over 12 hours. This low excretion rate appears to be specific only to man, because in other species much higher excretion rates have been found. In the pig, the rate of excretion has been found to be as high as 50-55% per hour (Dumont, 1955; Kay, 1963) and in cattle a 25% per hour elimination rate has been observed (Kraybill, Bitter and Hankins, 1952).

AP has been used extensively in laboratory animals and large animals to measure body water. Kraybill, Bitter and Hankins (1952) used it for estimating body fat in cattle, and reported a remarkably high accuracy for the method. McFadden and Richards (1956) measured body water in calves using AP and concluded that the method was suitable for determining body composition providing that gut-fill could be standardised. Garrett, Meyer and Lofgreen (1959) found the estimate of body water in ruminants by AP to be extremely variable and considered the method not accurate enough for nutritional investigations. They concluded that the variable amount of gut water, and the variable concentration of AP in the gut water, were the main factors responsible.

Reid, Balch, Head and Stroud (1957) found that AP entered the gut in appreciable amounts in steers and suggested that this was the reason for antipyrene overestimating the body water space in ruminants.

In pigs, Kraybill, Goode, Robertson and Sloane (1953) found that the AP space was useful for predicting the amount of separable fat in the carcass. A high excretion rate was observed (36%/hour), and it was also discovered that the drug became bound to plasma proteins, as shown by its variable recovery rate from the blood. Huckabee (1956) also found the drug to be bound to plasma proteins.

Other species in which AP has been used, are rabbits, dogs and monkeys (Soberman, 1950; Reid, Balch and Glascock, 1958) and goats (Panaretto, 1963).

The lack of precision of the AP method for estimating body water is possibly due to its rapid diffusion into abnormal fluid depots, and its small degree of lipid solubility (Soberman et al., 1949). The subsequent failure of many workers to emulate the results of Kraybill, Bitter and Hankins (1952) has stimulated interest in other compounds, some of these being derivatives of antipyrene.

(iii) 4-amino-antipyrene. Huckabee (1956) suggested the use of 4-amino-antipyrene (4-AA) for measuring body water in preference to antipyrene because of its more precise recovery from blood. Furthermore, 4-AA exhibited a lower lipid solubility than antipyrene and was more evenly distributed in the organs and tissues of dogs, goats and man. It was found to be freely diffusible between plasma and red blood cells but was totally excluded from lipid material. Administration of 4-AA to 35 human subjects showed that there was an initial rapid fall in the plasma concentration in the first hour, followed by a slow constant decline over six hours, averaging 6% per hour. The volumes of distribution of 4-AA and AP were determined simultaneously in eight subjects. The 4-AA space was found to be slightly smaller than the AP space (mean 4-AA space/AP space = 0.99) possibly because of the greater exclusion of 4-AA from lipid material.

In a comparison of three indirect methods of estimating body water in pigs weighing 27 kg, Kay, Jones and Smart (1966) found that the estimates of empty body water determined by 4-AA were variable and were not significantly correlated with the values obtained by chemical analysis. The rate of elimination of 4-AA was found to vary from 7 to 22% per hour in the pig.

(iv) N-acetyl-4-amino-antipyrene. In the course of investigations into the intermediary metabolism of AP, Brodie, Berger, Axelrod, Dunning, Porosowski and Steele (1951) studied the properties of N-acetyl-4-amino-antipyrene (NAAP). It was found to be negligibly bound to plasma proteins, its rate of elimination was negligible and its uniform distribution in the various tissues and organs of the dog was closely related to the water content of the organ or tissue. Reid, Balch and Glascock (1958) compared the use of NAAP with that of tritium, and AP, for estimating body water in rabbits. It was found that whereas the tritium and AP spaces were not significantly different from the TBW of the animal, the NAAP space was not significantly different from the empty body water (TBW less the water of GI tract). Up to 200 minutes after injection, only a small proportion of the NAAP had penetrated into the gut, but at 296 minutes the concentration of NAAP in the gut water was the same as that in the blood. It was also found that the maximum recovery of NAAP from the empty body, urine and gut contents to be 379 minutes after injection, indicating that NAAP was slowly metabolised. The finding that (a) NAAP was not metabolised, (b) only small quantities of it entered the gut water in less than 200 minutes after injection, (c) it was uniformly distributed in the body including the gut contents about five hours after injection, showed that NAAP was useful in determining the TBW as well as empty body water. Of the three solutes compared in the estimation of empty body water in

90-kg pigs, Kay (1963) found NAAP to be the most satisfactory. The estimates of body water by NAAP tended to be lower (mean 1.2%) than the values obtained by direct analysis of the empty body. The elimination of NAAP from the body was found to be only 6%/hour. Shumway (1959) found a large variation in the NAAP estimates of body fat and considered it impossible to develop an accurate prediction equation with the available data.

(v) Deuterium oxide. The naturally occurring non-radioactive isotope of water, deuterium oxide (D_2O) has been used extensively to estimate body water in live subjects because it is considered in addition to tritium, to be the ideal test solute.

Deuterium oxide has been used in medical research for (a) the determination of T.B.W. and the turnover rates of water (Edelman, Olney, James, Brooks and Moore, 1952; Schloerb, Friis-Hansen, Edelman, Solomon and Moore, 1950; Moore, 1946), (b) comparing the volume of dilution with that of other tracers such as antipyrene (Faller, Bond, Pette and Pascale, 1955; Hurst, Schemm and Vogel, 1952) and urea (San Pietro and Rittenberg, 1953; Bradbury, 1961), (c) the determination of body water in pathological states such as oedema (Hollander, Chang and Co Tui, 1949) and (d) for investigating the exchange of D_2O ions with tissue hydrogen ions (Krogh and Ussing, 1936; Ussing, 1938; Edelman, 1952).

There have been very few investigations in which D_2O has been used for estimating body water in farm animals. Groves and Wood (1965) used D_2O for measuring changes in T.B.W. of young pigs. The water content of the body measured by D_2O, underestimated T.B.W. determined by desiccation, by 2.41%. It would be expected that an overestimate of T.B.W. to occur due to exchange with labile hydrogen ions, but the authors concluded that their result was due to the slow equilibration of D_2O with the water in the bladder. It was also found that the relationship between D_2O and

body water was closer when the weight of the animal at the time of final blood sampling was used as a measure of body weight ($r = 0.8185$) than the weight at the time of injection of the D_2O ($r = 0.8013$). The authors assumed that the loss of weight between the two weighings was an insensible one.

More recently D_2O has been used for estimating total body water and body fat in pregnant Blackface ewes (Foot, 1969). It was found that there was less than 0.5 kg difference between the actual weight of body fat and the weight estimated from D_2O space when this was within 0.5% of the TBW value.

(vi) Tritium. The radioactive isotope of water, tritium (HTO) has been used more extensively than deuterium oxide for measuring body water as more simple techniques are available for its estimation in biological fluids. Tritium gives overestimates of total body water similar to deuterium oxide because, it too, exchanges with labile hydrogen ions in the body.

In domestic animals, the method has been used in goats (Panaretto, 1963), steers (Shumway, 1959) and pigs (Kay, Jones and Smart, 1966). Shumway (1959) compared AP, NAAP and HTO for estimating the body fat content of steers. Correlation coefficients of +0.56, +0.78 and +0.79, respectively, were found between the estimated values and the fat content determined by chemical analysis. Panaretto (1963) found that the HTO space overestimated total body water by 0.8% in goats, after the HTO space had been reduced by an arbitrary 3%, to adjust for exchange with labile hydrogen ions during the equilibration period. Kay, Jones and Smart (1966) found that equilibrium of HTO in the pig occurred within two hours of injection. For pigs of 27 kg liveweight, HTO overestimated empty body water by 0.75%, but with 90-kg pigs, the overestimate amounted to

10% and a non-significant relationship between HTO space and empty body water, was found.

(vii) Extracellular water measurements. The measurement of extracellular volume is often desirable in studies in which a knowledge of the distribution of body water is required. Extracellular water has a complex distribution. It is mainly distributed throughout the interstitial spaces of tissues and organs, and it is also contained within discrete fluid systems of the body. These include the blood vascular system, the lymphatic system, the intestinal tract and the cerebrospinal fluid.

Changes in body hydration during growth, malnutrition, illness and fluid imbalances are often reflected by changes in the extracellular water. No ideal solute has been found for the measurement of extracellular water, and although no well-defined compartment is measured, the estimation of extracellular volume as an additional variable can significantly improve the accuracy of body composition determinations (McCance and Widdowson, 1951; Hörnicke, 1961). The ideal solute for the determination of extracellular volume is one that diffuses rapidly across the capillary membranes into the intercellular fluid spaces and yet is strictly barred from diffusion through the cellular walls. The diffusion into the extracellular space should be rapid compared to the excretion and metabolism of the substance.

The solutes which have been used to measure extracellular space may be classified into two types, electrolytes and non-electrolytes.

The electrolytes which have been most extensively used are sodium thiocyanate (Crandall and Anderson, 1934; Doxiadas and Gairdner, 1948; Flynn, Hanna, Long, Asfour, Lutz and Zobrisky, 1968), sulphate (Lavietes, Bourdillon and Klinghoffer, 1936), bromide (Wallace and Brodie, 1939; Brodie, Brand and Leshin, 1939) and thiosulphate (Gilman, Philips and Koelle, 1946). More recently, radioactive substances have been used,

sodium (Kaltreider, Meneely, Allen and Bale, 1941), chloride (Winkler, Elkington and Eisenman, 1943), bromide (Duffus, 1967) and sulphate (Walser, 1952).

Possibly the most widely used non-radioactive electrolyte for estimating extracellular volume is sodium thiocyanate. It equilibrates rapidly (Doxiadas and Gairdner, 1948) and its renal excretion is low, but it enters the red blood cells and the gastric mucosa. It has been found to bind to lipids (Rosenbaum and Lavietes, 1939) and proteins (Scheinberg and Kowalski, 1950) and in some pathological conditions, the volume it measures, approaches that of total body water because of the increased permeability of the cells (Doxiadas and Gairdner, 1948). In a recent investigation, Flynn et al. (1968) considered that thiocyanate overestimated the extracellular water space in pigs, but from the results obtained from young children they indicated that it might be a valuable tool for the diagnosis of early malnutrition. Sulphate and thiosulphate have been found to remain almost exclusively extracellular (Bourdillon and Lavietes, 1936), but these substances undergo rapid urinary excretion. Using radiosulphate (^{35}S) Walser (1952) and Walser, Seldin and Grollman (1953) found that it measured a volume similar to that of inulin in man and dogs. Walser, Seldin and Grollman (1953) found that the ratio of radiosulphate to inulin space in normal persons was 0.95 ± 0.11.

Radiosulphate is possibly the most reliable of the electrolytes for measuring extracellular volume. Its renal excretion is low and its estimation by counting is precise although in combination with other radioactive tracers, the efficiency of counting would possibly be severely reduced.

Several attempts have been made to measure the extracellular volume using large molecules such as sucrose, mannitol, raffinose and inulin. The most extensive study of the distribution volumes of various

solutes was made by Swan, Maddisso and Pitts (1954) using nephrectomised dogs. The distribution volumes of the non-electrolytes were found to be lower than those of electrolytes. Similar results have been reported in human subjects (Gamble, Robertson, Hannigan, Foster and Farr, 1953). The disadvantages of using substances of large molecular weight are that they require difficult analytical procedures, are slow to penetrate through the capillary walls and are rapidly excreted. Within these limitations, inulin has been found to give the most precise estimates of extracellular space.

(viii) Plasma and blood volume measurements. Blood is the most accessible of the physiological body fluids in all mammalian species, and its two main components, plasma and red cells are easy to separate.

Investigations into the determination of plasma and blood volume have shown that the differences in the estimates in animals of similar weight may have been due to variations in the body composition (Hansard, Sauberlich and Comar, 1951; Bush, Jensen, Cartwright and Wintrobe, 1955; Doornenbal, Asdell and Wellington, 1962; Talbot and Swenson, 1963). Gregerson and Rawson (1959) considered that blood volume was more closely related to the fat-free mass than to liveweight. Attempts to relate red cell volume to lean body mass have been encouraged by reports that oxygen consumption is closely related to cell mass (Gopalan, Srikantia and Venkatachalam, 1955; Miller and Blyth, 1952).

There are three types of tracer which have been used to measure plasma volume:-

(a) dyes,
(b) radioactive isotopes,
(c) substances of high molecular weight.

(a) Evans Blue has been used for several years for estimating plasma volume. The dye is lost from the bloodstream at an appreciable

rate in all species so far tested. Gregerson and Rawson (1959) and Talbot and Swenson (1963) found this loss to vary between 14% to 35% per hour in the pig. Courtice (1943) found in the dog and goat that 10%to 15% of the Evans Blue was lost per hour. The loss of Evans Blue from the circulation can be corrected for, by using an extrapolation procedure (Gregerson and Rawson, 1943) to calculate the volume of dilution at injection time.

Although there is no direct evidence that the dilution volume of the dye accurately measures plasma volume, the Evans Blue method has given consistent and repeatable results in the pig (Anderson, McDonald and Elsley, 1969) and there are reports to suggest that the method can detect changes in plasma volume due to different physiological states (Hytten and Paintin, 1963; Anderson, McDonald and Elsley, 1969) and differing body composition (Nicol, Thomson and McLean, 1968).

(b) Radioactive substances which have been used for measuring plasma volume are radio-bromine and albumin labelled with iodine-131 (^{131}I) (Fine and Seligman, 1943; Gibson, Seligman, Peacock, Aub, Fine and Evans, 1946). Reeve and Franks (1956) drew attention to the fact that albumin labelled with ^{131}I often gives unexpectedly low estimates of plasma volume. This was found to be due to the adsorption of ^{131}I onto the volumetric glassware during the analytical procedure. In dogs, Gibson *et al.* (1946) found that the albumin labelled iodine-131 and Evans Blue estimates of plasma volume agreed within $\pm$ 10%, the dye dilution method tending to give the largest estimate. The results obtained in this experiment suggest that it is possible that some of the Evans Blue could have become attached to the albumin, resulting in a lower recovery of the dye from the plasma. Schüttert, Steenberg, Standal and Lein (1968) recently used ^{131}I to estimate plasma volume in bacon pigs and found the estimates of blood volume from

^{131}I and haematocrit values useful for assessing body fatness.

(c) Iron dextrans have also been used to estimate plasma volume. These are substances of high molecular weight (approximately 400,000) which are not normally present in the blood. McKenzie and Tindle (1959) were the first workers to use this method successfully and they claimed that the iron dextran method ("Imferon") had several advantages over the Evans Blue method, because it was less elaborate and did not suffer from interference due to lipaemia. In a comparison between Imferon dilution and Evans Blue dilution in twelve adults, the estimates of plasma volume made by Imferon tended to be smaller than those from Evans Blue.

Methods of estimating red cell volume have involved labelling the erythrocytes in vitro and subsequently measuring the extent of dilution of the label after injection. Red blood cells can be labelled with either carbon monoxide or the radioactive isotopes of iron, phosphorus and chromium.

The carbon monoxide method involves either the inhalation of a known quantity of carbon monoxide or the injection of previously saturated donor blood (Root, Roughton and Gregerson, 1946). Consistently higher estimates of red cell volume determined by carbon monoxide have been found in human subjects (Nomof, Hopper, Brown, Scott and Wennesland, 1954) compared with the chromium 51 dilution method. These workers found that significant quantities of carbon monoxide left the bloodstream during the mixing phase, which resulted in a lower concentration of carbon monoxide at equilibrium.

Radioactive iron, ^{55}Fe or ^{59}Fe, is incorporated into the haemoglobin of the erythrocytes and this can be achieved only in vivo. A blood donor is therefore required so that in vivo labelling can take place. The

labelled cells are removed from the donor and used on the test animal. This method requires the cross-typing of the blood of the donors and test animals (Gibson et al., 1946).

Radioactive phosphorus (^{32}P) has frequently been used to label red cells. A donor animal is required because the labelling of the red cells can only be achieved in vivo. Radiophosphorus is lost slowly from the erythrocytes. Reeve and Veall (1949) reported a loss of 6% per hour in man, and Nickerson, Sear and Reeve (1953) reported a similar loss in the dog. In the pig, Hansard, Sauberlich and Comar (1951) reported a loss of 10-12% per hour. These results indicate the necessity for a correction to be made after injection by extrapolating back to zero time (Gregerson and Rawson, 1959). Estimates of red cell volume in the pig, made by ^{32}P indicate that it increases with increasing bodyweight (Bush, et al., 1955; Hansard, Sauberlich and Comar, 1951), although not proportionately, and this can possibly be accounted for, by the increasing proportion of "inactive" lipid tissue in the body. In 90-kg pigs, Hansard, Sauberlich and Comar (1951) calculated the RCV to be 25.9 ml/kg liveweight, whereas Bush et al. (1955) found this to be 22.5 ml/kg liveweight and suggested the difference between the results of their study and those of Hansard, Sauberlich and Comar (1951) to be due to the relative obesity of the pigs used.

In 1950, Sterling and Gray reported the ability of radioactive chromium (^{51}Cr) to tag red blood cells and to measure red cell volume. ^{51}Cr has a long half-life (24.5 days) and is available as a high specific activity product which emits soft X-rays. The ^{51}Cr technique has been used extensively by Doornenbal and his co-workers. In his developmental work, Doornenbal (1962) used rats, and attempted to relate red cell mass to lean body mass. In this experiment he equated the chemically-determined protein

with lean body mass. The relation between red cell mass determined by ^{51}Cr, and the weight of protein was close (r = 0.96), but because there was a wide range in the liveweights, there was also an extremely close relationship between liveweight and the body protein (r = 0.99). In a second study with ten bacon pigs (Doornenbal, Asdell and Wellington, 1962), the red cell volume determined by ^{51}Cr was not found to be closely related to the weight of protein in the carcass. Three faults can be aimed at this work. Firstly, carcass protein was considered synonymous with the lean body mass. Secondly, the calculated weight of protein formed only a proportion of the total body protein because the head and viscera were discarded at slaughter. Thirdly, there was a range of liveweights (190 to 246 lb) which confounded the relationship between red cell volume and lean body mass. In a later study which involved 88 pigs, ranging in liveweight from 9-103 kg, the effect of liveweight was minimised by restricting the calculation of the regression equations to small weight ranges. In the weight range 81-103 kg it was found that blood volume explained 8% of the variance in the weight of protein in the carcass, and liveweight explained 58%. The authors concluded that although bodyweight was a major influence in explaining the variation in total body protein, the precision in the prediction of total body protein could be improved by the use of blood volume determinations.

(ix) Potassium 42. Potassium 42 (^{42}K) has been used extensively in medical research for estimating body potassium in both normal and abnormal physiological states.

In many studies exchangeable potassium (K_e) has been found to be poorly correlated with bodyweight (Talso, Miller, Carballo and Vasquez, 1960; Boling, Taylor, Entenman and Behnke, 1962) because of the variability in the amount of body fat. However, Talso et al. (1960) have found the relationship between K_e and lean body solids, and carcass nitrogen in rats to be remarkably constant (r = 0.99) from birth throughout life. In

human subjects of various weight and age groups, the correlation between lean body mass and K_e was also close ($r = 0.945$). Boling and Lipkind (1963) have shown that neither K_e nor exchangeable sodium was closely correlated with total body water, but the sum of these two constituents did show a high correlation with total body water ($r = 0.978$). Muldowney, Crooks and Bluhm (1957) related K_e and chloride space to lean body mass, red cell mass, and creatinine excretion. K_e was found to agree with these variables over a wide range of age and bodyweight ($r = 0.90 - 0.91$), and were the same for both males and females, agreeing with the work of Talso et al. (1960).

More recently Pfau (1966) estimated K_e in four pigs ranging in weight from 90-100 kg, using ^{42}K. He reported a good agreement between the fat-free body mass determined by carcass analysis and K_e. However, the factor he used to estimate fat-free mass from exchangeable potassium seems to have been derived retrospectively from the analyses of his four pigs. Fries and Lynch (1968) estimated K_e in 32 cross-bred male lambs. The range in weights of the lambs was from 25-40 kg and the potassium was counted by means of its γ-radiation. The correlation between fat-free body weight and K_e was 0.86.

The short half-life of ^{42}K precludes its accurate measurement in the animal much beyond 48 hours and only recently has potassium 43 been used in potassium dilution studies. Potassium 43 has a half-life of 22.4 hours which allows for longer periods of study than ^{42}K (Johnson, Hartzuck, Zollinger and Moore, 1969).

Potassium-42 dilution offers a simple method of estimating body potassium in live animals and possibly in conjunction with sodium-24, it could give an extremely precise estimate of the body cell mass.

(x) Inert gas dilution. Gas dilution involves the measurement of uptake of a tracer substance which dissolves uniformly in body fat. In order that the fat tracer be distributed around the body it must be soluble to some extent in water. A gas with a high fat/water partition coefficient is therefore desirable.

The gas dilution principle was first applied by Behnke, Thomson and Shaw (1935) who measured the amount of nitrogen washed out from a subject in a pure oxygen atmosphere. Nitrogen is more soluble in fat than water, and thus the amount of nitrogen in the body depends to a great extent on the body fat. The volume of nitrogen obtained by this wash-out process, corrected for the nitrogen dissolved in non-lipid tissues, provides an estimate of the amount of body fat. Many factors contribute appreciable errors to this method. Apart from the difficulty of correcting for pulmonary nitrogen, the presence of nitrogen in intestinal gases and absorption of nitrogen through the skin must be taken into consideration.

Subsequent attempts to measure body fat have involved the use of anaesthetic gases and the inert rare gas, krypton. Cyclopropane, which is a relatively safe anaesthetic gas has been employed in a number of studies for the estimation of body fat (Lesser, Blumberg and Steele, 1952; Kumar, 1957; Lesser, Perl and Steele, 1960; Lesser and Zak, 1963). Cyclopropane appears to have many advantages which make it suitable for gas dilution studies. It has a high fat/water partition coefficient of 52:1 (Blumberg, La Du, Lesser and Steele, 1952), is non-toxic and is relatively cheap to use. The major disadvantage with cyclopropane is that in human subjects and presumably in large domestic animals the equilibration time is long, amounting to some 12 hours (Lesser and Zak, 1963), although in rats it has been reported (Lesser, Blumberg and Steele, 1952; Kumar,

1957) to be only $1\frac{1}{2}$-2 hours. It is impractical to keep a human subject confined in a closed circuit with an anaesthetic gas for any length of time, and thus an extrapolation procedure is usually adopted (Lesser and Zak, 1963; Lesser, Perl and Steele, 1960) to estimate the equilibrium value. This procedure introduces errors into the estimate of body fat.

More recently, krypton, an inert gas, has been used for the estimation of body fat (Davidsson, MacIntyre, Rapoport and Bradley, 1956; Lesser and Zak, 1963; Hytten, 1964; Hytten, Taylor and Taggart, 1966). Only in one instance has the stable form of the gas been used (Lesser and Zak, 1963); in other investigations a radioactive form of the gas has been used, Krypton-85 (^{85}Kr).

Attempts by Hytten (1969) to measure body fat in pigs by ^{85}Kr dilution were not successful, due to difficulties in the administration of the gas.

Although ^{85}Kr seems to be the only inert gas used for measuring body fat, it is not known how other inert gases would behave. Xenon-133 appears to be an ideal gas as its partition coefficient between olive oil and water at 37°C is 22.4 (Ladefoged and Anderson, 1967) which is approximately twice the value for the olive oil/water partition coefficient of 8.78 (Yeh and Peterson, 1963; Hytten, Taylor and Taggart, 1966) reported for krypton.

Other techniques available for estimating body composition in the live animal

(1) Body density. In the measurement of body density it is assumed that the animal consists of two distinct components — fat and the fat-free mass, each having a constant density. The variation in the proportion of these two components can produce a range of body density extending from about 1.1 g/cm^3 to less than 1.0 g/cm^3 — a range of 10%, for which the fat varies from 1-2% to 50% of the bodyweight.

Body density is calculated as the bodyweight divided by the body volume. There are two main approaches to the measurement of body volume, air displacement or helium dilution.

In the first of these two methods an animal introduced into a chamber displaces a certain amount of air, the volume of which can be measured by pressure differences. The readings for pressure must be taken with great precision, and corrections must be applied for temperature and humidity. Proposals and recommendations for the estimation of body composition in farm animals by air displacement have been put forward by Blaxter (1955), who considered that the amount of gas in the gut contents would be the greatest source of error in the method.

Since that time air displacement has been attempted by several workers. Hix, Pearson and Reineke (1964) reported an excellent agreement ($r = 0.99$) between the volumes and densities of men and women obtained by air displacement and helium dilution. Kodoma and Pace (1963) reported a correlation of 0.84 between the specific gravity of live hamsters, determined by air displacement, and the specific gravity of the eviscerated carcasses obtained by underwater weighing.

The second method, the helium dilution procedure, is the most common gasometric procedure for the determination of *in vivo* body density. The low water solubility of that gas means that only a small proportion of it is lost across the alveoli into the lungs (Lawrence, Loomis, Tobias and Turpin, 1946). Siri (1961) has made this method a practical proposition. His contribution has been the accurate measurement of helium concentration, and the incorporation of corrections for relative humidity and temperature. The helium dilution method has been used for measuring the density in infants (Foman, Jensen and Owen, 1963), men and women (Hix, Pearson and Reineke, 1964) and in bacon pigs (Gnaedinger, Reineke, Pearson, Van Huss, Messel and Montoy, 1963; Kay, 1963). Gnaedinger *et al.* (1963) obtained a poor

correlation ($r = -0.22$) between body density determined by helium dilution and percent fat in the whole body. They indicated that the reason for the poor agreement was the problem of correcting for humidity and temperature, and movement of the animal inside the chamber.

The accuracy of the *in vivo* body density methods suffers because of the difficulties in measuring the changes in volume, pressure and humidity with a high degree of precision. In addition, the single body component other than fat which would be expected to influence body density is bone. The variation in the bone content of the body, which has a higher density than fat (3.0 as against 0.90 g/ml), must be considered. Buck, Harrington and Johnson (1962) showed that in pig carcasses there was a large variation in the muscle/bone ratios at constant fatness, although the muscle/bone ratios had little effect on carcass specific gravity when compared with muscle/fat ratios. Holme, Coey and Robinson (1963), Whiteman and Whatley (1953) and Whiteman, Whatley and Hillier (1953) also failed to show significant effects from muscle/bone ratios on the specific gravity of carcasses, and Joblin (1966) suggested from his data, that the coefficient of variation of the muscle/bone ratio needed to be of the order of 11% before a significant effect on specific gravity was shown. Pearson, Purchas and Reineke (1968) considered, that on evidence from the data of Morales, Rathbun, Smith and Pace (1945) the variation in bone density contributes little error to the estimation of body fat by body density because it comprises a relatively small proportion of the body.

(ii) Potassium 40 measurements. Potassium 40 (^{40}K) accounts for most of the naturally occurring radioactivity in living organisms, and potassium (K) isolated from various sources is reported to have the same isotopic composition (Vinogradov, 1957). ^{40}K has a half-life of 1.33×10^9 years and has a peak photon energy of 1.45 MeV.

The first determinations of body K by means of its natural radioactivity were reported by Burch and Spiers (1953) who used a large high-pressure ionisation chamber. These workers calculated the K content of man to be 0.21% ± 0.01% of the bodyweight. Miller and Marinelli (1956) noted a sex difference in the K content of the human body. They found K to be 0.188 ± 0.006% of liveweight for males and 0.154 ± 0.003% for females. These results are much lower than those of Burch and Spiers (1953) and may possibly reflect the different age groups, or different muscular builds, analysed.

Woodward, Trujillo, Schuch and Anderson (1956) using a Los Alamos human counter, correlated ^{40}K activity to the gross bodyweight and to the fat-free body mass. Fat-free mass was estimated from the total body water (tritium space) using the approximation that total body water is 72% of lean body mass (Keys and Brožek, 1952). They found that the variation between fat-free mass and ^{40}K activity was much less than the variation between gross bodyweight and ^{40}K activity.

The initial research on human subjects concerning the relationship between ^{40}K activity and body composition, led several workers in the animal sciences to recognise the ^{40}K method as a rapid and non-destructive technique for determining the amount of lean meat present in carcasses and in live animals.

Kulwich, Feinstein and Anderson (1958) counted the γ-rays emanating from intact hams in a Los Alamos whole body counter. The correlation coefficients between the ^{40}K activity measured as γ-rays/sec/lb of ham and the percent fat-free lean and the percent fat were 0.983 and 0.966, respectively (Table 2.1). It must be emphasised that in this investigation only two pairs of hams were studied which had only a small range in composition.

Kirton, Pearson, Nelson, Anderson and Schuch (1961) counted the ^{40}K activity of ground pork and lamb samples in uniformly sized cartons. The correlations between the percent K and the chemical components in both the lamb and pork are shown in Table 2.1.

Table 2.1

The relationships between ^{40}K measurements and body components from several studies

Author	y	x	r	±Syx %
Kulwich, Feinstein and Anderson (1958)	Pig hams			
	Fat-free lean	γ/sec/lb	0.983	-
	% Fat	"	-0.966	-
Kulwich, Feinstein and Golumbic (1960)	Pig hams			
	EE %	cpm	-0.99	2.2
	Protein %	"	0.99	0.64
	Water %	"	0.99	1.7
Kulwich et al. (1961a)	Pig hams			
	Sep. lean lb	cpm/lb	0.96	0.38
	" " %	"	0.87	2.5
	Sep. fat lb	"	-0.96	0.37
	" " %	"	-0.86	2.8
Kirton et al. (1961)	Ground pork (38 lb)			
	Water %	^{40}K(%K)	0.98	1.61
	EE %	"	-0.98	2.20
	Protein %	"	0.96	0.61
	Ground lamb (38 lb)			
	Water %	^{40}K(K%)	0.92	3.20
	EE %	"	-0.91	4.35
	Protein %	"	0.88	0.89
Kirton and Pearson (1963a) using Flame Photometry	Ground pork			
	% H_2O		0.997	0.54
	% Fat		-0.986	0.38
	% Protein		0.986	0.38
	% K from ^{40}K		0.977	0.01
	Ground lamb			
	% H_2O		0.993	0.97
	% Fat		-0.990	1.46
	% Protein		0.975	0.42
	% K from ^{40}K		0.936	0.01
Judge et al. (1963)	Lamb carcasses			
	Edible portion	cpm/lb	0.74	3.45
	Excess fat	"	-0.79	4.16
	Live lambs			
	Edible portion % lw.	"	0.75	1.42
	Excess fat % lw.	"	-0.74	1.39

The authors concluded that for the pork samples greater accuracy was obtained when the counting times were increased which would increase the difficulty of fitting the ^{40}K technique into a continuous meat-processing system as suggested by Anderson (1959).

Kirton, Pearson, Porter and Nelson (1961) measured the ^{40}K activity of ten newly-shorn Blackface lambs in a 4 π liquid scintillation counter. The animals were counted before and after washing the shorn fleeces. After counting, the lambs were slaughtered and dissected into carcass and non-carcass components. A difference in K concentration of 0.94 g K/kg liveweight between the washed and unwashed sheep was found, which was highly significant. The K content of the separable lean as estimated from ^{40}K was calculated to be 2.98 g K/kg. In addition, it was found that washing with detergent decreased the values of the correlations between the K content of the live animal and the various carcass components.

Kirton and Pearson (1963a) measured the K content of ten dressed lamb carcasses (including bone), 20 samples of ground pork, and 15 samples of ground lamb by ^{40}K activity and by flame photometry. For each sample it was found that K determined by flame photometry had a lower standard deviation than from ^{40}K. Correlations of 0.94 and 0.98 were found between the estimates of K determined by flame photometry and by ^{40}K activity for the ground pork and lamb, respectively. The relationships are shown in Table 2.1.

Judge, Stob, Kessler and Christian (1963) concluded that the ^{40}K activity of lamb carcasses could be used to predict carcass composition (Table 2.1) although it was found that relatively simple measurements of the longissimus dorsi or back-fat were equal or better in their predictive accuracy. In live lambs, no consistently significant relationship between the edible portion of the carcass and the whole body K content was found (Table 2.1).

(iii) Feed conversion ratio measurements. The feed conversion ratio of bacon pigs expressed as the amount of food consumed per unit weight gain, is the performance parameter which gives an indication of the efficiency of the animal as a convertor of food into body tissue. The fatty tissues and lean tissues which contribute to liveweight gain are dissimilar in their heats of combustion, the former being about five times as great as that of fresh lean tissue. It would therefore be expected that the feed conversion ratio be closely related to the percentage of fatty tissue in the carcass. Several studies have shown, however, that there are quite poor relationships between feed conversion ratio and the percentage of fatty tissue in the body (McMeekan, 1940; Lucas and Calder, 1956; Lucas, McDonald and Calder, 1960). One of the main confounding effects arises from the differences in the overall maintenance cost of the pigs taking different lengths of time to reach slaughter weight. In addition some notable studies have shown (Lucas and Calder, 1956; Lucas, McDonald and Calder, 1960) that reducing the growth rate of pigs results in a greater proportion of lean in the carcass, thus giving a low caloric density. The expected improvement in feed conversion ratio is counterbalanced by the increase in the overall maintenance cost.

The relationships between feed conversion ratio and the energy cost of maintenance and fat and protein deposition in the carcasses of growing pigs have been studied by Kielanowski (1966). He derived equations which could be used for estimating the content of protein and fat in liveweight gain when the feed conversion ratio was known. Although several assumptions were made in the derivation of these equations they did help to explain how the reduction in the fat content and the increase in the feed conversion ratio had been achieved in the Danish Progeny Testing Stations during the period 1926-27 to 1962-63.

The measurement of feed conversion ratio as a method of predicting body composition in the live animal has been used only once. Fowler (1966) presented data obtained from 77 castrate and female pigs in which measurements of feed intake, and growth rate and several indices of carcass fatness were made. The relationships between feed conversion ratio adjusted for maintenance, and the indices of carcass fatness were significantly higher than those involving the unadjusted feed conversion ratio. It was concluded that the corrected feed conversion ratio concept was useful for predicting carcass composition from feed intake and growth data alone.

Summary

A review of some of the methods for estimating body composition in living animals has been given. It can be seen that the basis of many of these methods relies on the assumption that measurements of part of the body can be used to predict the composition of the whole body.

It is obvious that many of the indirect methods can only be applied in specific situations in which there is the expertise and necessary equipment for implementing the techniques involved. Although a number of the more sophisticated techniques have been shown to be accurate in their prediction of body composition, many of the simpler methods are attractive because of their cheapness and ease of application.

CHAPTER 3

COMPONENTS OF ERROR IN PREDICTING BODY COMPOSITION

Introduction

The problem of estimating body composition in live animals can be resolved into three parts.

(a) The technical problem of obtaining an accurate estimate of liveweight.

(b) The assumptions about the inter-relationships between body components.

(c) The technical problem of measuring a component of the whole animal.

The magnitude of the errors of measurement of body components (part c) depends to some extent on the amount of time and money spent on each measurement (Harrington, 1963). Technical errors can be reduced, but according to Siri (1956) "there would still remain in most methods for estimating body composition, a residual uncertainty of about $\pm$ 4% of the body weight". Awareness of this could save effort being directed towards improving the precision of any technique which would produce only a marginal improvement in the accuracy of estimate of a body component.

The variability in the liveweight of the animal

One of the most important parameters in body composition studies is liveweight. Although it is the simplest of body measurements to record, it does not have a constant relationship with the empty body weight of the animal.

In many investigations, the errors in the prediction of body composition are due, in part, to the variability in the composition and quantity of the contents of the intestinal tract. This is an important

factor especially in ruminant studies in which the contents of the intestinal tract contribute to a large proportion of the liveweight. Results from the literature show that in sheep, alimentary fill can range from 11 to 21% of the liveweight for wethers (Taylor, 1954), 7 to 10% for ewes (Kirton and Barton, 1958) and 10-22% for steers (Kay, Macdearmid and Macleod, 1970).

In non-ruminants such as pigs, the problem of intestinal fill is less important. Reid (1956) has stated that in the pig the intestinal contents make up approximately 3.2% of the bodyweight and seldom exceed 5%. The proportion can of course be reduced by fasting. Kirton, Gnaedinger and Pearson (1963) found that the intestinal contents made up approximately 2% of the bodyweight in pigs which had been starved for 24 hours. Obviously in the estimation of the constituents of live animals, the variability of the contents of the intestinal tract will contribute substantial errors, particularly when the estimates are obtained by difference.

For several dilution techniques, the composition of the intestinal contents also contributes errors in the prediction of body components. The composition of the contents of the intestinal tract can be affected by the type of the diet and the degree of fasting. Water is normally the largest constituent of the intestinal tract of many species. In an extensive study Cizek (1954) calculated the proportion of gut water to total body water for several animal species, in different states of fasting. It was found that there was a general decrease in the proportion of gut water after deprivation of food and water for 48 hours.

In those studies in which dilution techniques are used for estimating body composition, body lipid is calculated as the difference between the <u>total</u> body weight and the fat-free weight, and thus in leaner animals the percentage error in the estimation of the weight of body fat is

greater than in more obese animals.

The non-absolute nature of the assumed biological relationships

Several attempts have been made to demonstrate the constant proportionality between various components of the bodies of animals of several species, so that a determination of one component permits the estimation of other components. In its simplest form, the body is considered as a two-component system consisting of fat and a fat-free mass, and a single measurement, such as body water, specific gravity or total body potassium is sufficient to determine the composition of the whole body. Analysis of the two-component system shows that in some instances large errors can result in the prediction of the fat-free weight and body fat from the estimation of any of the components of the fat free mass. This is because the fat-free mass is not an homogenous entity but consists of water, protein and mineral matter, the proportions of which vary either with age (Spray and Widdowson, 1951) or with body weight (Wedgwood, 1963).

Indeed, the data for pigs obtained by Clawson, Sheffy and Reid (1955) showed that over a wide range of body fatness (9 to 50%) and age (26 to 300 days) a curvilinear relationship between the percentage body water and the percentage body fat content was more precise than the straight forward application of a linear regression equation. This indicates that the water content of the fat-free mass is not constant relative to age, and this may limit the accuracy of certain in vivo techniques.

More recently Wedgwood (1963) calculated from the data of Rathbun and Pace (1945), Morales, Rathbun, Smith and Pace (1945) and Pace and Rathbun (1945) that there was a statistically significant variation in the density of, and the percentage of water in the fat-free eviscerated

carcass of the guinea-pig, as the weight of the fat-free carcass increased. From the regression equations which were calculated it can be seen that assumptions made about the constancy of the fat-free mass of animals in a population in which there is a wide range in body size, could lead to substantial errors in the prediction of body composition, using dilution techniques or density methods.

In an investigation into the use of specific gravity as a method for predicting carcass composition in pigs, Adam and Smith (1964) found that the muscle/bone ratio, in addition to the muscle/fat ratio was also correlated with carcass specific gravity, but to a much lesser extent. Nevertheless, when the muscle/bone ratio was included in the regression equations for the prediction of the percentage lean in the side from carcass specific gravity, there was a 12 to 20% increase in the accuracy of prediction.

This information in conjunction with that obtained by Buck, Harrington and Johnson (1962) who found that muscle/bone ratios differed between breeds and sexes, suggests that prediction equations based on carcass specific gravity, should be calculated separately for breed and sex.

The data from these studies indicate that, as a population of animals is divided into increasingly homogenous groups, the relationships between the body constituents will become less variable. Similarly, it can be postulated that as the fat-free body of an animal is divided into its component parts there will be less variability in the relationship between the components. It is with this background that several workers in this field have proposed multicomponent models of body composition which have helped to explain the variation in several constituents with increasing body weight (McCance and Widdowson, 1951; Anderson, 1963; Wedgwood, 1963).

In a number of techniques available for predicting *in vivo* body composition, only part of a body component is measured, and assumptions are made about the distribution of the remainder of the component. One example of this is the estimation of body fat by ultrasonics. A large proportion of the lipid fraction of the body is found in the subcutaneous adipose tissue and thus the measurement of the backfat thickness would seem to be a good measure of the total fat content of the body. However, the variation in the fat depth at one site in relation to depths at other sites, and the variation in the size of the internal fat depots in relation to the amount of subcutaneous fat, contribute substantially to the relatively low accuracy of the method for predicting total body fat.

Summary

The sources of error which can arise in the prediction of *in vivo* body composition have been discussed.

Apart from the technical problem of measuring a component of the whole animal, errors can arise because of (a) the variability in the live-weight of the animal (b) the non-absolute nature of the assumed biological relationships.

The effect of (a) is particularly evident in those circumstances in which the weights of body components are obtained by difference from liveweight. The contributory effect of (b) depends not only on the type of measurement made, but also on the homogeneity of the groups of animals studied.

Part 2

THE BACKGROUND TO THE PRESENT INVESTIGATION AND THE FORMULATION OF THE EXPERIMENTAL PROGRAMME

CHAPTER 4

THE RELEVANCE OF PAST STUDIES TO CURRENT PROBLEMS IN ANIMAL PRODUCTION REQUIRING THE MEASUREMENT OF BODY COMPOSITION

Introduction

The studies on body composition reported in the literature can be grouped into three types. The classification depends on which of the following aspects contributed the main subject of the investigation:-

(a) Fundamental relationships between body components.

(b) Development of new techniques and methodology for assessing the size of a body component.

(c) The actual application of theoretical knowledge and methodology to animal production and scientific situations requiring an assessment of body composition.

The state of progress in body composition work may be judged by the extent to which studies in categories (a) and (b) have facilitated the development of (c). The agricultural significance of the developments in each of these fields is summarised below.

Fundamental relationships between body components

There is an extensive literature describing the relationships between body components for many species, covering a wide range of normal and abnormal physiological states. For species of agricultural importance, such documentation is relatively scarce and much of the fundamental knowledge gained has been secondary to the main objectives of the studies. The reason for this, is perhaps that most agricultural research tends to be mission-orientated, with the immediate problems of the industry over-riding the requirement for fundamental information. Short-term solutions cannot

always be adapted to new situations and in the long term, there is a great need for more basic studies.

The needs of the meat producing industry exemplify this. The weights at which animals are slaughtered are becoming more flexible, and in these circumstances, the growth process is intercepted at many different stages. Physiological relationships which hold at traditional slaughter weights may not necessarily hold at other stages.

Development of techniques and methodology

Much time and effort has been devoted to the development of techniques for assessing body composition in vivo. In the medical field the relevance of this research is obvious, since diseased states due to fluid and electrolyte imbalances, can be easily diagnosed by various methods, and changes in body composition during pregnancy and postnatal growth can be readily monitored. In agriculture, however, the relevance of such research is more limited. The applications are not primarily concerned with morbidity, and, though qualitative estimates may suffice in the medical application, in agriculture the requirement is for precise quantitative measurements. It is for this reason that the uncritical transference of medical techniques to agricultural application may prove disappointing. In addition, methods which are justifiable in human medicine in a life-or-death situation are not necessarily appropriate in the agricultural context. For example, tritium which is the long-lived radio-isotope of hydrogen is frequently used to determine body water in normal and oedematous human subjects. The use of tritium in an agricultural context is strictly limited to highly specialised research in which its use can be carefully controlled to prevent the indiscriminate contamination of the environment.

The application of in vivo techniques to practical and scientific situations

The final phase in integrating the concepts and the techniques,

is in applying them to a practical or research situation in which the measurement of body composition is required. The relevance of papers concerned with this aspect to the current agricultural requirements, is surprisingly small. There are very few animal studies in which special emphasis has been given to the rationale for choosing a particular indirect method or group of indirect methods for a given application. Much of the agricultural research in this context has been directed at demonstrating the effectiveness of only one method rather than setting out to establish the relative merits of several methods. Even when relative evaluations have been attempted, they have been often limited by the restricted facilities or expertise available at the one centre and so have not always examined a representative range of candidate methods.

Few studies have been specifically designed to compare the various indirect methods of assessing body composition for different levels of application. The element which is lacking in the literature is the type of data which will allow studies to be made on the cost-effectiveness of different techniques used singly and in combination. Studies are required in which several indirect estimates of body composition are compared simultaneously using identical animals. These should be ultimately slaughtered to provide absolute reference values for the compartments being estimated. Existing data tend to be restricted to the predictive value of a single measurement. In practice, it may prove much more fruitful to combine two or more relatively inexpensive predictors than to use one single expensive method, however good it appears to be in isolation.

This last point can be illustrated by using the analogy of a potential purchaser of a building, trying to assess its merits from external inspection only. A view through one window merely gives the impression of one room or compartment. The whole property may have many rooms or

compartments, and only by peering through several windows can a comprehensive picture of the interior be formed. Like the property, the animal has several compartments, such as fat, empty body water space, extracellular water space, lean body mass and blood.

From the analogy it is clear that the continual refinement of the accuracy of one method may not be as productive in predicting the composition of the animal as using other measurements which are different in type.

The objectives of the present investigation can be considered in terms of the analogy used above. The simultaneous application of several indirect methods to the same animal is similar to assessing the property by looking through different windows. One indirect method can give only one picture of the total composition, but as more indirect methods are applied, so a more complete picture of the composition can be obtained. The information accruing from each indirect method can be statistically combined to give the best possible estimate of any given body component.

The philosophy can be summed up by quoting the remarks of Francis D. Moore, speaking at a body composition symposium in 1963; he said "You don't need to measure more people, you need to measure more things in the people you measure."

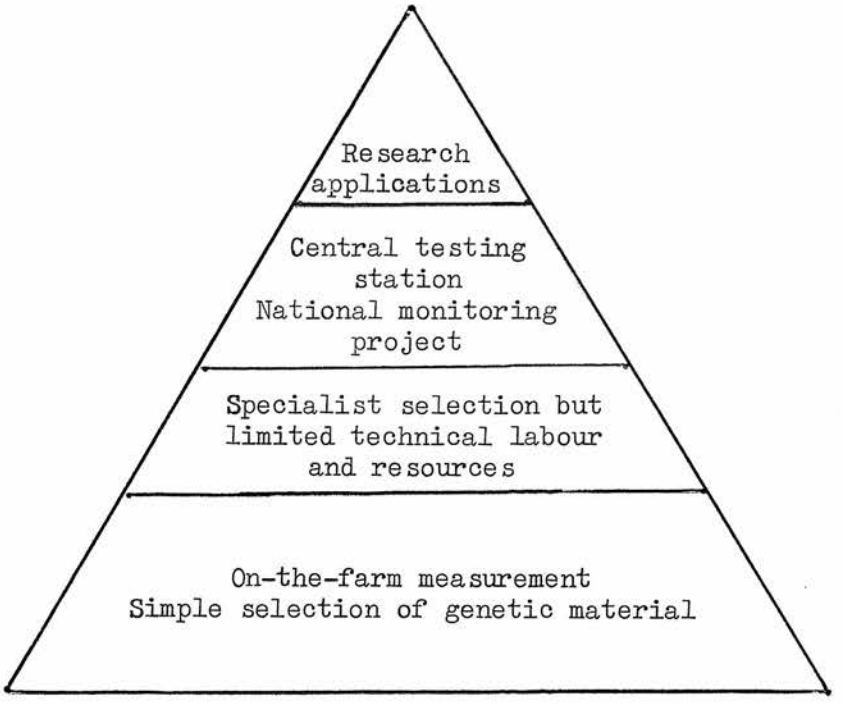


Fig. 5.1. Four situations related to animal production in which indirect techniques can be applied

CHAPTER 5

GENERAL BACKGROUND TO THE INVESTIGATION

Introduction

The main purpose of this study was to examine in detail the relative merits of different methods of assessing *in vivo* body composition in the pig. The limits were widely set so that both practical and research applications could be examined simultaneously.

There are many advantages in conducting this type of study with the pig since body composition has not only been of great significance in determining the profitability of pig production, but it is a species which has been extensively studied in biomedical research.

In formulating a programme of study, decisions were taken on which concepts were the most relevant for agricultural application. Similarly, the indirect methods studied, were chosen on the basis of their relevance to the agricultural industry and agricultural research. Routine agricultural applications may require only simple and cheap indirect methods whereas in specific research projects, the detection of relatively small differences in composition require the more complicated techniques.

Four specific situations related to animal production were considered. These are shown in Fig. 5.1.

The pyramidic shape illustrates the relative widespread nature of each of the four levels of research. At one extreme there are the relatively simple methods which can be used in a large variety of circumstances, and at the other there are the sophisticated methods utilising the extensive facilities of biological research centres.

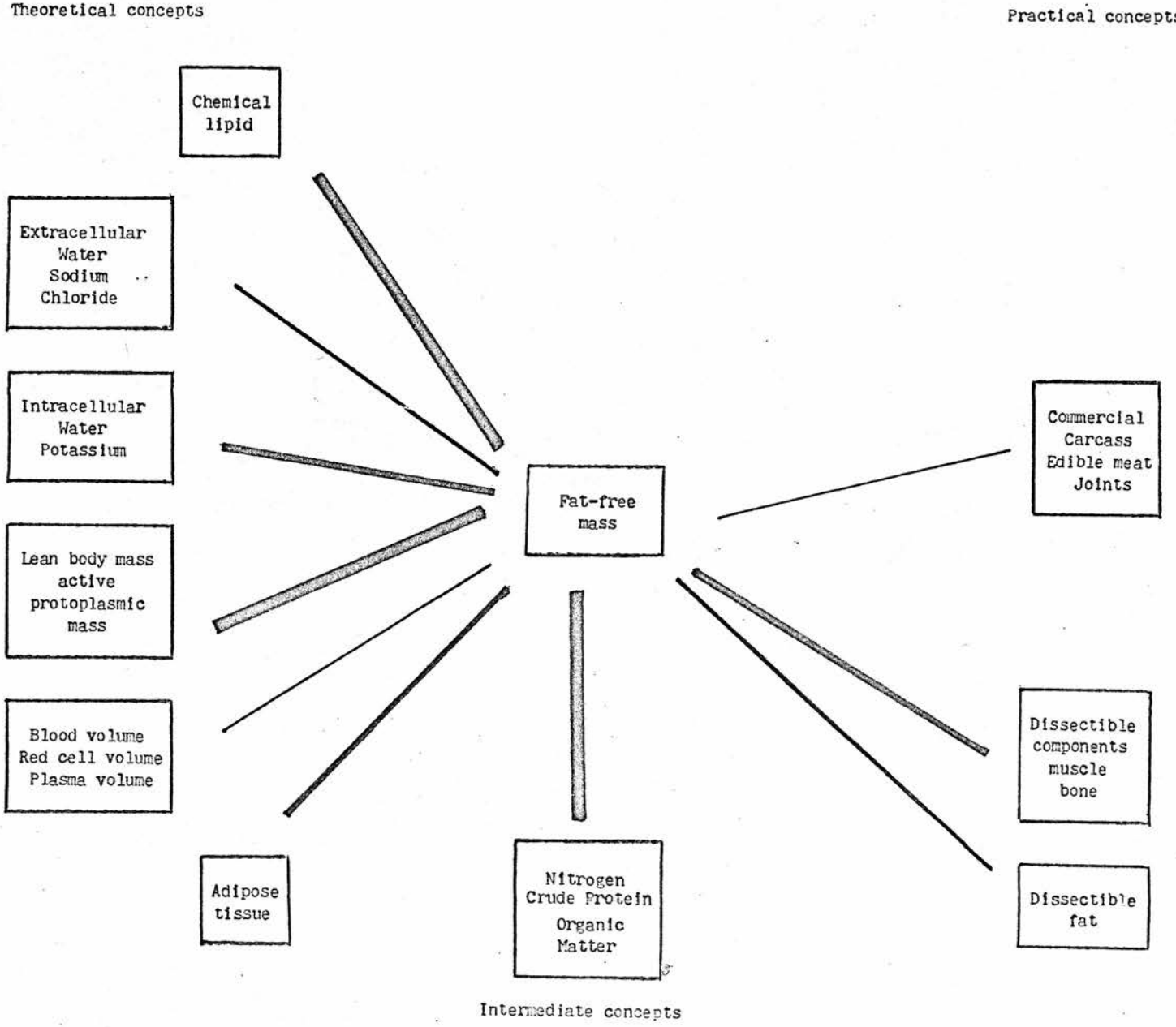


Fig. 5.2. Illustration to demonstrate the centrality of the fat-free mass concept

Choice of concepts used to describe the composition of the body

The different concepts used to describe body composition are convenient for qualitatively defining the compartments of the body which are of special interest in different types of investigations. From the literature it is clear that the concept of the fat-free mass has been selected as central to many themes of body composition study. Its immediate significance in many studies may not be apparent and therefore Fig. 5.2 was compiled to illustrate the centrality of the fat-free mass concept and its relationship to other body components.

Some of the components shown in Fig. 5.2 are not ideally suited for practical studies on body composition because of their heterogenous composition. For example, adipose tissue consists not only of lipid material but also of water and cell solids. The lean body mass includes small proportions of lipid material and the extracellular space is often defined as including the water of the intestinal and urinary tracts.

The chemical concepts of body composition are much more convenient to refer to, because of their homogeneity. The fat-free mass for example is defined as the lipid-free empty body, and body lipid is defined as the ether-extractable fraction of the empty body.

Choice of methods for estimating in vivo body composition

There are several methods available for measuring the various chemical and physical components of the live animal. Some of these methods are not readily applicable in most situations because of the requirement for specialised equipment and technical expertise. For example, many of the methods which are available for measuring in vivo composition are based on the use of radioactive tracers. These afford a simplicity and precision which the non-radioactive tracers seldom exhibit. However, the use of

radio-isotopes in animal studies gives rise to problems of residual radio-activity which limits their application. A number of the commonly-used radioactive tracers have long half-lives which places restrictions on carcass disposal. Another disadvantage is that in some instances, the methods of collection of samples and the analysis of radioactivity requires specialised equipment. These restrictions severely limit the suitability of tracers such as tritium, which has a half-life of 12.26 years, or krypton-85 which has a half-life of 10.6 years.

Facilities are not readily available at many biological research centres for the counting of the natural radioactivity of the body, emanating from potassium-40. This method requires specialised and costly equipment and difficulties are encountered in calibrating the instrument and allowing for the different geometry of varying body shapes. Krypton-85 has been used with some success for the direct measurement of body lipid in human subjects, but difficulties have been reported (Hytten, 1969) on the quantitative administration of the gas to pigs.

A more detailed description of each of the indirect methods which were chosen for estimating body composition, is given in the remainder of this chapter.

Estimation of the fat-free mass

(i) Potassium space. Potassium (K) has been reported to form a relatively constant proportion of the intracellular fluid space (Manery, 1954; Brožek and Henschel, 1961) and to be almost completely excluded from the extracellular fluid (Edelman, 1961). Several investigators (Woodward et al., 1956; Anderson, 1959; Forbes, Gallup and Hursh, 1961; Talso et al., 1960) have suggested that the K content of the body is a good index of muscle mass.

There are two methods of determining body potassium in live

animals; counting the γ-radiation emanating from potassium 40 (^{40}K) or by dilution technique using potassium 42 (^{42}K).

There are two main problems in measuring the ^{40}K content of live animals. Firstly there is the problem of the variability of the shape and size of different animals in the counting system, and secondly there is the problem of self-absorption of radiation within the animal. Despite the numerous attempts which have been made to overcome these two problems (see Miller and Remenchik, 1963) the ^{40}K counting technique requires highly specialised equipment which was not available during the course of the present investigation.

It was therefore decided that the potential of the ^{42}K dilution method be fully explored. ^{42}K is a short-lived isotope (12.44 hours) and is relatively easy to determine in body fluid samples, (Haberer, 1965). It has been used extensively for the determination of exchange rates in different organs of several species of animal; (Noonan, Fenn and Haege, 1941; Levitt and Guadino, 1949; D'Silva and Neil, 1951; Ginsburg and Wilde, 1954; Harris, 1952, Walker and Wilde, 1952) and for the prediction of body composition in human subjects, (Talso et al., 1960; Boling and Lipkind, 1963; Boling, Taylor, Entenman and Behnke, 1962; Muldowney, Crooks and Bluhm, 1957; Corsa, Olney, Steenburg, Ball and Moore, 1950), rats (Talso et al., 1960), sheep (Fries and Lynch, 1968) and pigs (Pfau, 1966).

The distribution of ^{42}K in the body after its intravenous injection has been studied by many workers. It has been found that more than 95% of the injected ^{42}K leaves the plasma in the first two minutes (Walker and Wilde, 1952) and some organs rapidly acquire a higher specific activity than others, because of their different rates of potassium turnover. Walker and Wilde (1952) have classified the organs of the body into four groups with respect to the exchangeability of potassium, and

several workers (Ginsburg and Wilde, 1952; Sheppard and Householder, 1951) have studied the kinetics of the system by compartamental analysis. It has been found that the labelling of the most slowly equilibrating organs proceeds exponentially towards parity with the plasma, indicating that all the body potassium is exchangeable. By solving the plasma specific activity time-course equation, and with a correction for urinary ^{42}K loss, the whole body specific activity can be determined, and thence the total body potassium. This refinement is probably not justified as it requires several measurements of the plasma specific activity, and in addition some workers (Corsa et al., 1950; Talso et al., 1960) have found that at 24 hours the proportion of tissues which have not fully equilibrated form only a small proportion of the total lean body mass. Thus the 24 hour exchangeable potassium can be expected to be correlated with total body potassium.

^{42}K has been used mainly in human subjects for predicting body composition in which no absolute verification of the accuracy of the method has been possible, although it has been found to accord well with other indirect methods. In rats, correlations of over 0.98 have been found between the total exchangeable potassium measured by ^{42}K dilution and the fat-free carcass solids or carcass nitrogen (Talso et al., 1960). However, the more recent work of Fries and Lynch (1968) did not generate much optimism for the method. The correlation between ^{42}K space and the fat-free mass was low ($r = 0.86$). This was possibly because the labelled potassium had never fully equilibrated with the body potassium because food had been given during the equilibration period. In addition, ^{42}K lost in the faeces during the equilibration period was not accounted for, although it was later found to amount to about 10% of the dose given.

These results indicate that the ^{42}K dilution technique is useful

for the determination of the fat-free mass in the live pig.

(ii) Deuterium oxide space. Although many non-radioactive tracers have been extensively used for the measurement of body water, deuterium oxide (D_2O) was chosen for this study because it is considered the ideal body water tracer, and has been reported to have all the advantages of tritium as well as being non-radioactive.

The differences in the physical properties of D_2O and water are such that small changes in D_2O concentration can be measured by several methods. The properties of D_2O compared with those of ordinary water are shown in Table 5.1.

Table 5.1

The physical properties of water and deuterium oxide (after Urey and Teal, 1935)

Property	H_2O	D_2O
Density	1.0000	1.10764
Temperature at maximum density	3.98	11.23 ± 0.02
Molar volume at temperature of maximum density	18.015 cc	18.140 cc
Refractive index	1.33300	1.32828
Viscosity in millipoises		
10°C	13.10	16.85
20°C	10.09	12.60
30°C	8.00	9.72
Melting point	0.000	3.802
Boiling point	100.00	101.42

Deuterium oxide is not toxic in small doses and according to Hevesy and Hofer (1934) and Hurst, Schemm and Vogel (1952) it is metabolised in the same way as ordinary water. Deuterium has been found to exchange with labile non-aqueous hydrogen (Ussing, 1938) with the result that total

body water (TBW) has been overestimated by up to 5% (Forbes, 1962; Moore, 1946) or 1-2% of bodyweight (Hevesy and Jacobsen, 1940; Schloerb, Friis-Hansen, Edelman, Solomon and Moore, 1950).

The equilibration times of D_2O in different species of animals varies, as the data in Table 5.2 show.

Table 5.2

Equilibration times for D_2O in several species of animal

Species	Equilibration time	Author
Man	1-3 hours	Forbes (1962)
Guinea-pig	3 mins	Flexner, Gellhorn and Merrell (1942)
Rat	60 mins	Haigh and Schneiden (1956)
Dog	32 mins	Montgomery and Fogelman (1950)
Pig	5 hours	Groves and Wood (1965)
Sheep	4 hours	Foot (1969)

The estimation of D_2O in body fluid samples is a complex two-phase procedure. The first phase involves the distillation of the fluid sample to obtain pure water/D_2O mixtures without fractionation taking place (Turner, Neely and Hardy, 1960; Schloerb, Friis-Hansen, Edelman, Sheldon and Moore, 1951). The second phase involves the measurement of the D_2O concentration in the purified mixtures. This can be achieved in several ways: Refractive Index measurements using an interferometer (Rittenberg and Schoenheimer, 1935; Keston, Rittenberg and Schoenheimer, 1937), Pycnometer (Robertson, 1949), Falling Drop technique (Fenger-Erikson, Krogh and Ussing, 1936; Cohn, 1947; Hytten, Taggart, Billewicz and Jason, 1962), Infra-red spectrophotometry (Stevens and Thurston, 1954;

Turner, Neely and Hardy, 1960) and neutron bombardment (Haigh, 1953).

The measurement of small concentrations of D_2O in biological fluids has been made by both the Falling Drop technique and by Infra-red spectrophotometry at this Institute (Foot, 1969). It was found that the Falling Drop technique was extremely laborious, but the Infra-red spectrophotometric method was relatively simple to operate and did not demand the same rigid environmental conditions.

D_2O has given precise estimates of body water in human subjects (Brinkman, 1965), in young pigs (Flynn et al., 1968; Groves and Wood, 1965) and in sheep (Foot, 1969), and it appears to be a promising technique for the estimation of body water in growing pigs.

(iii) Urea space. The inclusion of the urea dilution method into this investigation was based on reports (San Pietro and Rittenberg, 1953; Bradbury, 1961) which favourably compared the method in terms of its accuracy of estimation of T.B.W. in human subjects with the deuterium oxide dilution technique. Its main advantage over D_2O is its very low cost and its ease of determination in biological fluids.

Urea equilibrates with the body water fairly rapidly (Bradbury, 1961). The method of dilution is such that in most instances in the first hour after injection the urea concentration in the blood declines exponentially, but thereafter the rate of decline remains relatively constant. If it is assumed that the rate of excretion is the same during the mixing period as in the latter part of the equilibration period, the initial concentration (which would be obtained if complete distribution were instantaneous or if none of the tracer left the body) can be determined by extrapolation of the straight portion of the curve back to the time of injection (T_o) (Painter, 1940; San Pietro and Rittenberg, 1953; Bradbury, 1961).

The rate of equilibration and the rate of disappearance of exogenous urea have been found to be independent of the dose given (Painter, 1940). In all calculations of urea space, corrections must be made for the initial urea concentration.

Estimation of the active protoplasmic mass

It has been well established that the oxygen consumption of mammals depends on the amount of metabolically-active tissue in the body and thus high correlations between the total blood volume and the red cell volume, the oxygen carriers, and the active protoplasmic mass, the oxygen consumer, can be expected.

Evans Blue dilution. Evans Blue dilution was used in this study to provide estimates of the blood volume in conjunction with haematocrit measurements. It is a simple method, involving the quantitative injection of the dye into the blood-stream, followed by the frequent sampling of the blood, to establish a disappearance curve (Anderson, 1967). The method has been applied with some success to adult pigs (Anderson, McDonald and Elsley, 1969).

Evans Blue has been reported to disappear from the blood stream at an appreciable rate for all the species so far tested (Gregerson and Rawson, 1959; Talbot and Swenson, 1963; Anderson, McDonald and Elsley, 1969). Talbot and Swenson (1963) found this loss to vary from 14% to 35% per hour in pigs. An extrapolation procedure (Gregerson, 1943) corrects for this loss of dye from the blood-stream, and the volume of dilution at injection time (To) can be calculated. Rawson (1943) has attributed the slow rate of disappearance of the Evans Blue to its preferential binding to the albumin fraction of the plasma protein. Several workers (Gregerson and Rawson, 1959; Talbot and Swenson, 1963) have reported the rate of loss of Evans Blue from the blood stream to be constant, and to conform to a

first order reaction, but a more recent study by Anderson (1967) has shown a variable rate of decline of Evans Blue in sows. Anderson (1967) has stated that calculations based on approximation to a first order reaction can result in an overestimation of plasma volume of 5 to 10%.

Estimation of the extracellular water space

Changes in body hydration during growth, malnutrition and after the ingestion of large quantities of fluid are mainly changes in the extracellular fluid volume. Measurements of body water using indirect techniques often fail to account for the varying hydration of the body, and the determination of extracellular volume has been reported (McCance and Widdowson, 1951; Hörnicke, 1961) to improve the accuracy of body water determinations.

Sodium thiocyanate. There are many reports on the use of organic and inorganic tracers for the measurement of extracellular volume, and it is obvious that some are more accurate than others but involve complicated analytical techniques.

For this reason, a non-radioactive tracer, sodium thiocyanate, was chosen because it is a simple technique and the analytical procedure is not complicated (Bowler, 1944). Sodium thiocyanate equilibrates in the body in the same time as D_2O (Doxiadis and Gairdner, 1948) and in conjunction with D_2O, a determination of the true intracellular water space can be made because it also measures the transcellular and red blood cell water.

Certain precautions must be observed in the analytical procedure. Bowler (1944) reported that appreciable fading occurs if the addition of ferric nitrate to form a colour complex with the thiocyanate is made in strong light. He suggested artificial light and the immediate measurement of the colour intensity after addition of the ferric nitrate.

Estimation of dissectible fat

(i) Ultrasonics. There are many reports in the literature concerned with the ultrasonic appraisal of body composition in farm animals. The method was included in this study because it has been shown to be easy to operate, and in conjunction with an experienced operator provides information on the thickness of subcutaneous fat at various points which are known to be indicative of the body fat content.

Much of the developmental work with ultrasonics has involved measuring the dimensions of the longissimus dorsi (Price, Pearson and Emerson, 1960; Stouffer, Wallentine and Wellington, 1959; Stouffer, Wallentine, Wellington and Diekmann, 1961) and relating these to the lean content of the carcass. There are also several studies which have been concerned with the prediction of fat in the live animal from ultrasonic measurements of backfat at various anatomical points (Kleisch et al., 1957; Hazel and Kline, 1959; East et al., 1959; Price et al., 1958; Alsmeyer, Hiner and Thornton, 1963).

The closest relationships between backfat thickness, determined by ultrasonics and various carcass measurements have been obtained using an operating frequency of 2.5 mc/second (Hazel and Kline, 1959; East et al., 1959) with repeated measurements at the same point (East et al., 1959). Almost all studies have reported the restraint of the animals, while the ultrasonic readings have been made, although Stouffer et al. (1961) considered the movement of the animal to be unimportant because their probing device was attached to the animal's back. Cook and Cuthbertson (1967) concluded that operator "experience and interest were necessary to take accurate readings". Stouffer et al. (1961) stated that the ultrasonic fat measurements in pigs were more accurate than in cattle because of the greater fat depth in pigs, which would allow greater

discrimination between the outgoing signals and the echoes. However, it has been suggested (Price et al., 1958) that errors are increased with increasing fat thickness because the calibration of the oscilloscope scale assumes that the velocity of sound through fat is the same as that through lean. Goldman and Heuter (1956) reported that the speed of sound through muscle was 10% more than through fat.

Changes in the carcass measurements due to slaughter are considered to affect the accuracy of ultrasonic measurements for predicting backfat depth. Hedrick, Meyer, Alexander, Zobrisky and Naumann (1962) suggested that there may be a change in the carcass contours during the slaughtering procedure resulting in real differences between pre- and post-slaughter dimensions. Lauprecht, Scheper and Schroeder (1957) found that fat measurements taken on the live animal were more closely related to measurements on the chilled carcass in the standing position, than those on the hanging carcass. Hanging the carcass is thought to compress the longissimus dorsi at both ends, resulting in an expanded cross-sectional area at the last rib. There is also variation in the cross-sectional area of the eye muscle, depending on whether or not it is measured above a rib or intercostal space. In a more recent investigation (Ramsey, Williams, Hobbs, Cole and Temple, 1965) it was concluded that the effect of the slaughter procedure on muscle and fat configuration was not a major source of error in ultrasonic evaluations.

(ii) External measurements. There have been numerous attempts made to relate the external dimensions of pigs to body fatness. Schmidt, Forsthoff and Winzenburger (1935) reported that relative chest depth (chest depth expressed as a percentage of height at withers) was related to fat/lean ratio in some breeds of pig, but Brüggemann (1940) did not find such close relationships in other breeds of pig. Hogreve (1942) demonstrated

that the ratio of the length (occipital bone to the base of the tail) to the chest girth could be used as a rough measure of fatness. Harrington (1958) suggested that because these German investigators were concerned with pigs suitable for fat production, it would be difficult to demonstrate relationships between the carcass quality and body dimensions in the lean type of pig required in the bacon-producing countries.

External measurements were included in the study because it was considered that they may possibly yield some information on the composition of the body in those situations in which expensive equipment and technical expertise are not available.

(iii) Visual assessment. Visual assessment is the simplest and cheapest in vivo measurement of body composition. It is often used in farming circles for the appraisal of meat animals. However, there have been only a few attempts made at assessing the value of visual appraisal of live animals. Willman and Krider (1943) obtained a correlation of 0.42 between 'condition' as determined by visual appraisal of 268 live pigs and their carcass backfat thickness. Harrington (1958) suggested that the significant correlation possibly arose because of the presence of extreme values. Bratzler and Margerum (1953) tested the accuracy of three experienced judges in predicting body length and backfat and the preferred-cut yield in 434 pigs ranging in weight from 180 to 240 lb. The correlations of these three characters with the judges' predictions were of the order of 0.6, 0.5 and 0.3, respectively, although there were some differences between the judges in the degree of accuracy of their predictions. The authors suggested that considerable training and experience would be necessary before live pigs could be accurately assessed so that carcass grades could be related to the appraisal of the live animal.

Despite the subjective nature of visual appraisal in estimating body composition in live animals, it was included in this study as it is

obviously applicable in some situations in the lower layers of the pyramid (see Fig. 5.1).

Estimation of total body lipid

Feed conversion ratio. The studies of Kielanowski (1966) and Fowler (1966) have indicated that the measurement of feed conversion ratio adjusted for the energy requirements of maintenance is closely related to the caloric density of the carcass. This measurement was included in the present study because it is a simple and inexpensive technique but it may give relatively accurate estimates of body composition.

Post-mortem measurements

Several body composition measurements were made on the carcasses of the experimental animals soon after they had been slaughtered. Obviously the application of these measurements can only be made in those situations in which the tested or experimental animals are eventually killed.

(i) Estimation of body lipid by specific gravity. Many reports in the literature indicate that the differences in the densities of fat and lean can be used to estimate the proportions of these components in the carcass by a determination of the specific gravity (Adam and Smith, 1964; Joblin, 1966; Holme, Coey and Robinson, 1963; Whiteman and Whatley, 1953; Whiteman, Whatley and Hillier, 1953). Although Buck, Harrington and Johnson (1962) considered that the variation in the muscle/bone ratio in addition to the variation in fat content could also affect specific gravity determinations, several other workers have failed to show significant effects of muscle/bone ratios on specific gravity (Joblin, 1966; Whiteman and Whatley, 1953; Whiteman, Whatley and Hillier, 1953).

Specific gravity measurements are simple to obtain and the equipment and expertise were already available because it is a routine carcass measurement in the Institute.

(ii) Estimation of dissectible fat by backfat measurements.

Backfat measurements taken on the chilled carcass side allow a comparison to be made between the operators in their accuracy of measurement of backfat thickness by ultrasonic and, of course, provide information on the amount of subcutaneous fat and its distribution.

The extensive investigations of McMeekan (1941) and Hankins and Ellis (1934) showed that fat measurements taken at certain points on the beck were useful in assessing the amount of fat in the carcass. These fat measurements were taken at the shoulder, mid-back, loin and over the eye muscle.

The experimental programme

The project was divided into two phases. The first part was devoted to the development and application of five methods of estimating body composition in the pig, and the second, to a relatively large-scale experiment in which several measurements were taken on 24 specially prepared animals. In all, three experiments were carried out and brief details of these are shown in Table 5.3 which shows the overall plan of the investigation.

Table 5.3

Overall plan of the investigation

Phase 1	Experiment 1	Experiment 2
	Developmental Study	Exploratory Investigation
	^{42}K dilution	Urea dilution
	D_2O dilution	Thiocyanate dilution
		Evans Blue dilution
	20 castrated male pigs, 90 kg	2 mature sows
	Comparison with chemical analyses of the carcasses	

Phase 2 — Experiment 3

Simultaneous application of indirect methods to each animal

^{42}K dilution	External measurements	Feed conversion
D_2O dilution	Ultrasonic measurements	
Evans Blue dilution	Visual appraisal	

24 female and castrated male pigs, 90 kg
Comparison with chemical and physical analyses of the carcasses

In the first phase of the project, experiment 1 was concerned with the development of the potassium-42 method for the estimation of the fat-free mass, and the deuterium oxide technique for the estimation of body water. Experiment 2 was an exploratory investigation into the use of the urea, sodium thiocyanate and Evans Blue dilution techniques. Some of the methods attempted in this experiment have been applied to other species with some success, but have never been used in the pig for the specific purpose of predicting body composition. The main object of this experiment was to acquaint the author with the analytical techniques involved in the application of these methods, and at the same time to assess the suitability of each method for its inclusion into the second part of the study.

The satisfactory experience gained in the application of each method in the first part was a necessary prelude to its inclusion in the second phase of the investigation. In experiment 3, seven methods were applied simultaneously to each animal so that an accurate compositional assessment could be made using different combinations of methods. Comparisons between the indirect estimates of the body components and the physical and chemical components of the carcasses were made.

Statistical considerations

In experiment 3 it was considered that the only possible valid comparison of the numerous methods which could be made at this scale of operation, was by applying them to pigs at a constant liveweight. This was to overcome a problem which has arisen in many previous studies in which indirect estimates have been compared with direct estimates of body composition. High correlation coefficients have been often found between X, the value of an indirect measurement and Y, the weight of a body component which was being assessed. Harrington (1963) highlighted the problems which are associated with the interpretation of such correlation

coefficients. He gave many examples from the literature showing that when Y was correlated with X, much of its variation could be attributed to the variation in the liveweight of the animal. Similarly, it was frequently shown that X was only of value in predicting Y, because of its close relation to liveweight.

In the present study, the methods were applied to each animal at 90 kg liveweight. This weight was chosen because it is the marketable weight of most pigs in this country, and also because it corresponds to the prepubertal stage when they are selected for their potential breeding worth.

Part 3

EXPERIMENTAL SECTION

CHAPTER 6

DETAILS OF THE EXPERIMENTAL STUDIES

Introduction

In this chapter, details of the experimental studies are given, which include details of the animals, their housing and management during the growing period, and their management during the *in vivo* studies. Details are also given of the method of slaughter of the animals, their subsequent carcass processing and the chemical and physical analyses. The chapter concludes with the descriptions of the several *in vivo* techniques which were used for estimating body composition.

Description of the animals, housing and management

In each of the three experiments, Large White x (Large White x Landrace) pigs were used, which were obtained from the Institute's hysterectomy-derived pig herd. During the course of these experiments, no overt signs of enzootic pneumonia were evident.

The management of the pigs from birth to weaning was similar throughout all the investigations. Iron was given at 2 to 4 days of age, and castration of the male pigs took place at 3 weeks of age. The pigs were weaned at 6 weeks, and creep-feed was available from 2 weeks to 6 weeks of age, and also for a further two weeks. At 8 weeks the pigs were transferred to a standard diet, NRS1, the composition of which is shown in Appendix Table 1. During the experimental period the pigs were housed individually in either a controlled-environment wing of the piggery or group-housed in a Harper Adams type of unit, and were individually fed twice daily. Increments of feed intake were made for

each pig, every 7 days, after they had been weighed. The pigs were weighed at the same time every week to minimise the variation in the gut contents.

In Experiment 1, 20 castrated male pigs were used. They were reared on different growth curves from 25 kg to about 90 kg liveweight. The design of experiment 1 and the dietary treatments imposed are given in Chapter 7.

In the second experiment only two sows were used. The absolute results of the in vivo techniques were not required because the experiment was specifically designed to investigate and compare certain dilution methods.

In the third experiment 12 castrated males and 12 female pigs were used. They were reared on different growth curves from 25 kg to about 90 kg liveweight. The design of this experiment, and the dietary treatments imposed, are given in Chapter 9. The management of the pigs from 25 kg to 90 kg was similar to that in experiment 1, except that when each pig approached 90 kg liveweight it was given a standard feed intake for seven days. This was an attempt to reduce the variation in the gut contents before the indirect techniques were applied.

Management of the pigs during the in vivo studies

In each of the three experiments, immediately before a pig was confined to a metabolism cage, it was weighed. In experiment 1 each pig was starved of food and water for 12 hours before the body water measurements were made. This was intended to minimise the contents of the bladder and the intestinal tract. In the second experiment the animals were weighed before the measurements were made, and were usually deprived of food and water for about 12 hours prior to the application of the indirect methods. In experiment 3 each animal had full access to food and water up to the

time of confinement, after which only water was available, except prior to, and during the body water measurements.

In order to obtain frequent and uncontaminated blood samples, the venous catheterisation method described by Anderson and Elsley (1969) was used on each of the pigs in the three experiments. These workers reported that the technique was fairly simple; required no deep surgery; fluids could be injected into and withdrawn from the blood stream at will, and the catheter could remain patent for long periods of time.

A polyethylene catheter about 20" long (bore 0.75 mm) was established in the external jugular vein via a medial ear vein. Attempts at putting the catheter down lateral ear veins were usually unsuccessful. Anaesthesia was induced and maintained by trichloroethylene (Trilene ICI Ltd.).

When it was not in immediate use, the catheter was cleared first with heparinised saline (0.9% sodium chloride and 100 I.U. sodium heparin/ml) and then filled with concentrated heparin. A nylon fishing-line, used as a stylette, was then pushed down the full length of the catheter. Each time the stylette was removed, a small quantity of heparinised saline was injected to clear the catheter. During intermittent use a short stylette (about 6" long) was used, and the rest of the catheter was filled with concentrated heparin.

After the catheter was established each pig was put into a galvanised steel cage equipped for the collection of faeces and urine.

Samples of blood were withdrawn from each pig through the catheter into evacuated tubes (Becton Dickinson Ltd.). An initial sample of blood was taken and discarded because it was usually contaminated with either heparinised saline or sodium pentobarbitone.

In the third experiment, in which female pigs were also used,

it was necessary to separate the urine from the faeces for the purpose of analysis. A urinary catheter (Eschmann, England) was established in the bladder and the urine was collected in a polythene container. On a number of occasions, each pig had to be restrained in its cage to permit a delicate procedure to be performed, such as the injection of a tracer, adjustment of the catheter, or the replacement of bandages and tape of the ears. In these circumstances, 5 to 10 ml of a dilute sodium pentobarbitone solution was injected down the catheter to induce a mild anaesthesia, lasting about 10 minutes. This was practised as little as possible, so that there was the minimum of interference to the animal's metabolism.

Slaughter of the animals and subsequent processing

After the full complement of body composition measurements had been made, the animals in experiments 1 and 3 were killed. This was accomplished by injecting 10 ml of a concentrated sodium pentobarbitone solution into the blood-stream. After slaughter, the carcasses were processed in the following manner:-

When organs or pieces of the carcass were removed from the body, they were weighed individually and put into doubly-sealed polythene bags. These were stored for about five to six days at $-25^{\circ}C$ to allow the radioactivity emanating from ^{42}K to decay to a low level before being transferred to a non-radioactive area for further processing.

From this stage onwards, the method of processing the carcasses in experiments 1 and 3, differed. This is indicated in the text.

(1) After slaughter, the animals were suspended by the hind legs and weighed on a steel-yard to the nearest 20 g. The

major blood vessels of the neck were cut and each carcass was bled as fully as possible. The weight of blood was recorded.

(2) The carcass was then cut down the mid-ventral line and the entire alimentary tract removed, weighed, emptied and reweighed. The intestinal contents were mixed and sampled for further analysis. Similarly, for each pig the bladder was emptied, the volume of urine noted, and a sample retained for analysis.

(3) In experiment 1, after the internal organs were removed, each carcass was beheaded at the anterior edge of the atlas vertebra, and then cut into joints, each weighing about 7 kg, and stored in the manner described above.

In experiment 3, the carcass was subjected to physical dissection in addition to chemical analysis. The carcass was divided into three parts after the hair had been removed by electric clippers. The middle of the carcass, designated MX, included the head, backbone, blood, flare fat, internal organs, and the entire alimentary tract. The left-hand side of the carcass, designated PDS (Physically dissected side), was put into a doubly sealed polythene bag and stored horizontally for about 16 hours at $+1^{\circ}C$. Careful handling of this side was necessary to prevent distortion of the carcass contours. After various backfat measurements and a determination of the specific gravity had been made, the side was physically dissected into skin + subcutaneous fat, dissectible lean and bone.

These components were weighed and stored in the usual manner.

The right-hand side of the carcass, designated JS (jointed side), was jointed into pieces weighing approximately 7 kg. These were stored in the usual manner, after which they were chemically analysed.

(4) After the cold-storage period at -25°C the carcass components were allowed to thaw. Each component was then removed from the polythene bag, weighed and then minced. In experiment 3 the three divisions of the carcasses remained as separate entities throughout this process and throughout the chemical analyses. The mince was mechanically mixed and then sampled. About 1 kg of the homogenous mince was used for chemical analysis.

Methods of chemical analysis

The chemical analyses of the carcass material provided the direct estimates of the body components, empty body water, fat-free mass, total protein and total fat.

The analyses for ether-extract, nitrogen, ash and certain constituents of the ash, were made on the dried carcass material which was obtained by freeze-drying the mince in aluminium trays for 24 hours at -45°C under 1 mm of mercury pressure. The residual dry matter content was determined by heating about 5 g of the freeze-dried material in an oven at 100° to 102°C. Drying was continued until the weight of the material became constant.

For the proximate analyses, the freeze-dried material was ground in a small hand-operated mincer, or in an electric coffee grinder. The

extremely fatty samples were comminuted by the former method. The proximate analyses were based on the A.O.A.C. methods (1965). Details of these methods and their modifications are given in Appendix 2.

Description of the methods used for estimating in vivo body composition

(i) Potassium 42 dilution

Experimental. The day after each pig was catheterised and confined to a metabolism cage, about 0.4mC of ^{42}K as potassium chloride in 10 ml of isotonic saline (The Radiochemical Centre, Amersham, Bucks, England) was injected down the catheter. The catheter was immediately flushed with heparinised saline and the stylette was re-introduced into the catheter. Immediately before and after injection the syringe and needle containing the ^{42}K were weighed.

About 0.2 to 0.4 ml of the same radioactive solution as that injected was weighed and diluted for use as a standard for counting. The extent of dilution was usually about 1:1000.

From the time of injection until slaughter, the urine and faeces which were voided, were collected and the ^{42}K activity determined by counting samples from each urination and from the total mass of faeces. Blood samples up to 24 hours post-injection were obtained from the pigs in experiment 1 so that a determination of the equilibration time could be made. In experiment 3, samples of blood were taken only after equilibration of the ^{42}K in the body had been achieved.

Counting procedures. ^{42}K has a half-life of 12.44 hours and disintegrates by producing both β and γ rays. The β rays have a mean energy of 3.6 Mev. and are reported to exhibit Čerenkov radiation (Haberer, 1965; Elrick and Parker, 1968). Čerenkov radiation is emitted from high energy β particles ($>$ 260 kev) which are slowed in passing

through an aqueous medium. The conditions required for measuring Čerenkov radiation have been described (Haberer, 1965; Elrick and Parker, 1968). The main condition is that the aqueous sample be optically clear, so that colour quenching by various pigments is negligible.

The measurement of Čerenkov radiation is a relatively simple procedure, and sample volumes up to 20 ml may be counted which require no scintillation medium. The measurement of Čerenkov radiation also permits a high efficiency of counting which was imperative in the present study because of the rapid radioactive decay of ^{42}K.

^{42}K was counted on a Packard Tri-Carb Liquid Scintillation Counter, Model 3324 (Packard Instrument Co.), which allowed the simultaneous measurement of each sample in three separately-adjustable channels. In each channel the gain could be varied between 0% and 100% and the window settings between 0 and 1000 divisions. Preliminary investigations with aqueous solutions of ^{42}K indicated that the optimum window and gain settings were 70-1000 divisions and 30%, respectively.

The count rate of each sample was recorded on computer cards and on a printed read-out. The information on the punched computer cards enabled the calculation of the activity of the particular sample to be corrected for radioactive decay. The total decay time for each sample was calculated from the time the original specific activity of the sample was determined (T_o), to the time of midpoint of counting.

Preliminary investigations into the counting of ^{42}K by Čerenkov radiation

The objective of these investigations was to determine the efficacy of the techniques for decolorising the plasma and urine samples in the preparation for counting their activity by Čerenkov radiation.

In the first test, blood plasma was deproteinised using a 10% solution of trichloroacetic acid (TCA). The ratio of blood plasma to

TCA was 1:1. To 5 ml of the deproteinised plasma, and to 5 ml of distilled water, 1 ml of an aqueous solution containing ^{42}K was added. Within the limits of counting error, the count rates for the two solutions were found to be similar.

In the second test, urine was decolorised using activated charcoal. The ratio of activated charcoal to urine was 3 g/30 ml. To 10 ml of the decolorised urine, and to 10 ml of water, 1 ml of an aqueous solution containing ^{42}K was added. As in the first test, the count rates for the two solutions were found to be similar.

In the final test, the linearity of response to graded additions of a high activity solution of ^{42}K to deproteinised plasma and to decolorised urine was tested. No deviations from linearity were found.

From this series of preliminary investigations the following procedures were developed for measuring the activity of ^{42}K in plasma and urine, by means of Čerenkov radiation:-

To 5 ml of plasma, prepared by centrifuging 10 ml of whole blood at 3000 r.p.m. for 15 minutes, was added 5 ml of freshly prepared 10% TCA. After shaking and centrifugation at 3000 r.p.m. for 15 minutes, 5 ml of the clear supernatant was pipetted into a 20-ml glass counting vial and diluted to 20 ml with distilled water.

Urine was decolorised by vigourously shaking 30 ml with 3 g of activated charcoal for two minutes. The mixture was filtered through a Whatman No. 9 filter paper. Ten ml of the filtrate was added to 10 ml of water in a glass counting vial.

The activity of the ^{42}K in faeces and gut contents was also estimated by counting the Čerenkov radiation. The samples were thoroughly mixed with water and then centrifuged. The supernatant was clarified with zinc sulphate as described by Hydén (1954).

The determination of potassium in the biological fluids. The determination of the specific activity of a biological fluid sample requires not only a knowledge of the radioactivity emanating from the isotope, but also of the amount of inorganic element which is associated with this radioactivity. The determination of potassium was made on the plasma and urine samples by flame photometry. In experiment 1, potassium was determined on a Carl Zeiss (Oberkochen) instrument; in experiment 3, an EEL flame photometer (Evans Electroselenium Ltd., England) was used. The mode of operation was the same for each instrument. The mutual interference effects between sodium and potassium were minimised by using standard solutions with different sodium/potassium ratios. Three calibration curves were constructed, the sodium/potassium ratios being 24:1, 33:1 and 40:1. Both the sodium and the potassium contents of the plasma and urine samples were measured, and by interpolation between the three potassium calibration curves, the appropriate correction for the interference due to sodium could be applied.

(ii) Deuterium oxide dilution

Experimental. In experiment 1, deuterium oxide (minimum purity of 99.7%) (Koch Light Laboratories Ltd.) was injected into the blood-stream of each pig as a 0.9% saline solution. The syringe used for injection was a 100-ml all-glass syringe with a luer lock. The syringe was weighed immediately before and after injection to the nearest 0.005 g. After injection of the D_2O, the catheter was filled with heparinised saline and a nylon stylette inserted the full length of the catheter. The dose rate for D_2O was calculated as that which would give a concentration of 0.1% to 0.2% of D_2O in the plasma.

In experiment 1 it was observed that leakages of D_2O often occurred between the plunger and barrel of the syringe, and between the

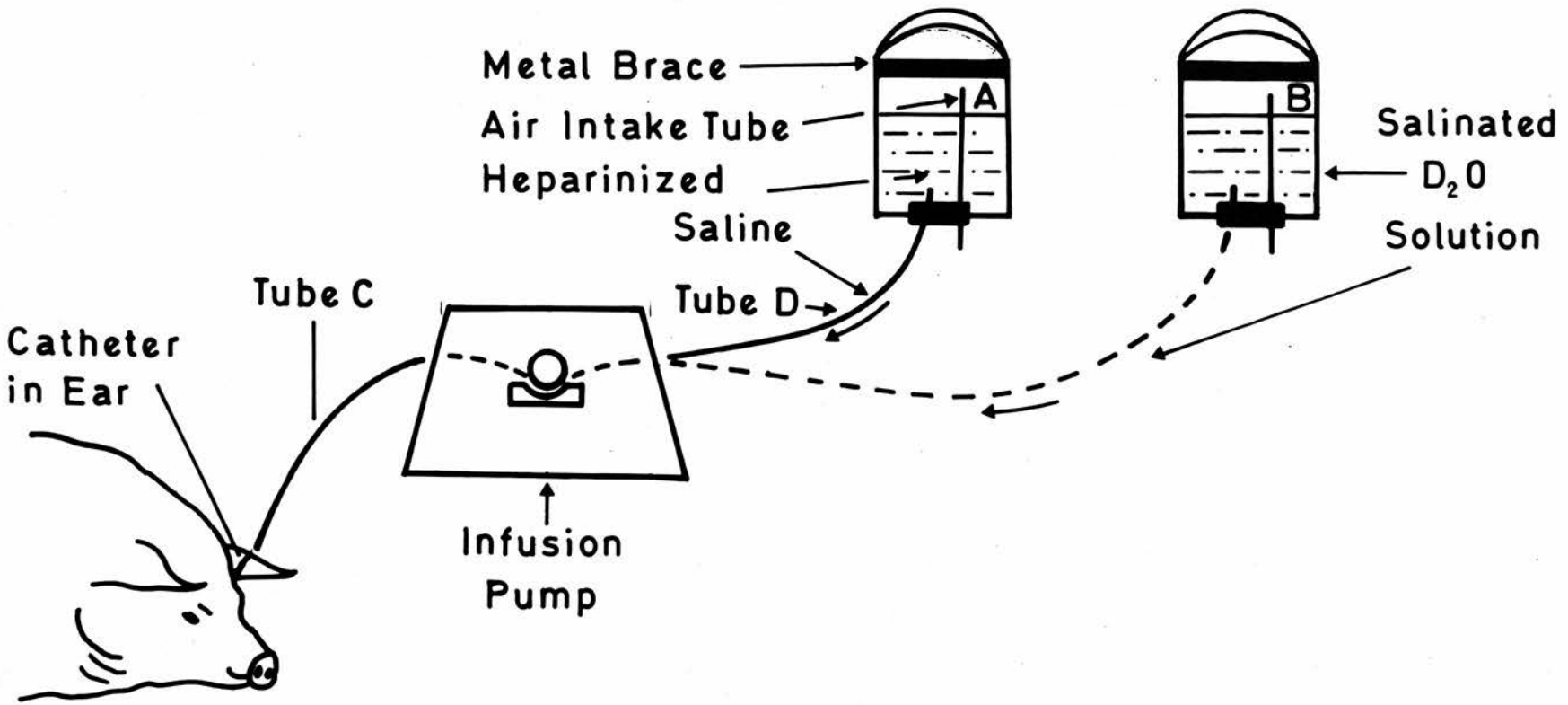


Fig. 6.1 Infusion of D_2O into the Bloodstream of the Pig by Peristaltic Pump

syringe and needle because of the large back-pressure. Fortunately, these losses of D_2O were recorded by weighing and a deduction was made from the total amount injected. In experiment 3, to minimise these losses, the D_2O was pumped into the blood-stream by a peristaltic pump (Watson Marlow Ltd.). The pumping system is shown in Fig. 6.1. Two infusion bottles each of which had a rubber cap and a metal brace at the opposite end, were used. Both bottles had an air intake tube so that liquid withdrawn from the bottles could be replaced by air. Initially, bottle B was weighed and then filled with a salinated (0.9%) D_2O solution. The bottle was then inverted and any liquid in the air-intake pipe removed. The bottle was mounted on a circular cork disc, so that further weighing could be facilitated in the inverted position (Wt 1). Prior to infusion of the D_2O, tube D, connected at one end to the intake side of the infusion pump, was connected to bottle A which contained heparinised saline. The pump was then switched on and heparinised saline was allowed to fill tubes C and D. Tube C, connected at one end to the delivery side of the pump, was then connected with the catheter in the ear vein by means of a needle. Tube D was then connected to bottle B, and the D_2O was pumped into the blood-stream. The length of time of delivery of 100 ml of D_2O was about eight minutes. Before all the D_2O in bottle B had been pumped out, the pump was switched off and tube D connected to bottle A. The pump was switched on again and heparinised saline was pumped down tubes C and D again, and any D_2O remaining in these tubes was pumped into the blood-stream. After infusion of the D_2O into the animal, bottle B was reweighed on its circular cork disc (Wt 2). The amount of D_2O injected was calculated as Wt1-Wt2 corrected for the amount of salt.

No values were available for the equilibration time of D_2O in bacon pigs, and it was assumed that this would be about the same as for tritiated water (Kay, 1963), that is, about two hours. However, in

experiment 1, the blood samples were collected at hourly intervals for about 10 to 12 hours, and in some cases sampling was continued for 24 hours. In experiment 3, after it had been established that equilibration occurred within two hours, blood samples were collected only up to four hours. Blood was obtained from the pigs by withdrawal through the catheter into evacuated tubes. It was then centrifuged at 3000 r.p.m. for 15 minutes in order to separate the plasma from the red cells. Urine and faeces were collected during the equilibration period and their volumes and weights recorded.

The estimation of D_2O in body fluids. The estimation of D_2O in biological fluids involves (a) the purification of the sample without isotopic fractionation taking place, and (b) the determination of D_2O in the water of the sample.

(a) In experiments 1 and 2, the vacuum distillation method described by Schloerb et al. (1951) was used. This method, which prevented isotopic fractionation of the water/D_2O mixtures was found to be laborious, and it required large amounts of "dri-ice". Sometimes it was found that the yields of purified water/D_2O mixtures from plasma were low, and for urine, the yields were nearly non-existent, an observation in agreement with that of Foot (1969). The glassware was also found difficult to clean, and assembling the apparatus for the distillation of six samples was time-consuming.

Before experiment 3 was started, another method of purifying body fluids was investigated. This method, described by Turner, Neely and Hardy (1960), involves heat in the distillation of the plasma samples. It had been used successfully by Foot (1969) with sheep serum and urine. The details of the method are given below:-

(1) About 0.3 g of anhydrous copper sulphate was added to 5 ml of the plasma sample and mixed thoroughly. The mixture was heated in a water bath at 90°C for five minutes.

(2) The sample was then removed from the bath and centrifuged at 1500 r.p.m. for five minutes.

(3) The supernatant was placed in all glass microstill to which heat was applied. The water/D_2O mixture was collected in a cold finger, distillation being complete within about five minutes.

Preliminary runs with plasma blanks were disappointing. In the first stage, in which anhydrous $CuSO_4$ was added to the plasma, a solid blue mass was formed which yielded a low quantity of supernatant on centrifugation.

The method was modified by adding 0.3 g of the anhydrous $CuSO_4$ to about 7-8 ml of plasma in a 1" diameter test tube, heating in a water bath as in the original method, but subsequently centrifuging at 3000 r.p.m. for 20 minutes, instead of 1500 r.p.m. for 10 minutes. This procedure gave a high yield of distillate in the plasma samples which were tested. With urine samples, however, the distillate contained compounds which, on introduction into the cells of the infra-red spectrophotometer, caused a rapid deterioration of the barium fluoride windows. It was therefore decided that for further application, urine would not be analysed, and its excretion during the equilibration period would be kept to a minimum by starving the animal of water.

The efficacy of the heat distillation method was tested. Standard solutions of D_2O were constituted with plasma solution (50% pig plasma:50% water) and purified by the heat distillation method. The

water/D_2O samples were then analysed on the infra-red spectrophotometer. The results are shown in Table 6.1.

Table 6.1

Recovery of D_2O from plasma samples purified by heat distillation

	% D_2O recovered	
% D_2O in sample	Replicate 1	Replicate 2
0.25	100.00	98.75
0.20	100.15	98.40
0.15	96.96	97.90
0.10	94.12	98.48
Mean	97.81%	98.38%
Standard deviation	± 2.48%	± 0.31%
Overall mean recovery	98.10 ± 1.79%	

It can be seen that the recoveries with the lower D_2O concentrations were not as good as with the higher D_2O concentrations. This was possibly due to the increasing error in pipetting small volumes of D_2O, and also to the reduced sensitivity of the infra-red spectrophotometer in the lower range of D_2O concentration. From this table it would appear to be advantageous to give the animals a dose of D_2O such that it gives a concentration in the plasma in the 0.20% region.

It was concluded that the modified method of heat distillation would be useful in these studies for the purification of plasma samples, and the method was used continuously in experiment 3.

(b) The second phase of the estimation of D_2O in biological samples involves the determination of the concentration of D_2O in water. In the three experiments, D_2O was determined by infra-red spectrophotometry as described by Turner, Neely and Hardy (1960) and more recently by Foot (1969).

Essentially the quantitative determination of D_2O by infra-red analysis, depends on the differential absorption of the infra-red radiation in the region 2500 cm^{-1}, between HDO and water. The infra-red spectrophotometer used was a double beam type (Unicam SP200). The cells were made of stainless steel and had barium fluoride windows. The sample cell was semi-permanent and the path-length was determined by the thickness of the neoprene spacers between the windows. The path-length was usually 0.100 mm. The reference cell had a variable path-length and was always filled with distilled water.

A preliminary investigation was conducted to determine the accuracy of measurement of D_2O using the infra-red spectrophotometer. The optical densities of a series of standards were obtained during a six-day period. Twelve readings were obtained for each standard. The standard error for the determination of D_2O in water was found to be $\pm$ 0.00084% at a D_2O concentration of 0.200%.

(iii) Urea dilution

In experiment 2, urea was used to measure total body water.

Prior to the injection of the urea, a blood sample was withdrawn from the animal. A determination of the urea content of this sample was made so that the level of endogenous urea at the time of dilution would be known.

A 40% urea solution was constituted from pure crystalline urea and sterile distilled water. It was injected into the blood stream through a catheter by an all-glass syringe. The injection took about 4 to 5 minutes. Immediately before and after injection, the syringe was weighed to the nearest 0.005 g. The dose rate of the urea was 150 ml/kg liveweight.

After injection, blood samples were collected every 15 minutes

for about 4 to 5 hours. Blood was withdrawn through the catheter into evacuated tubes and immediately centrifuged at 3000 r.p.m. for 15 minutes to separate the plasma from the red cells.

The urea in the plasma and urine samples was determined by a colorimetric procedure (Marsh, Fingerhut and Miller, 1965) on a Technicon Autoanalyser. Standard solutions of urea were similarly analysed. From each estimate of urea concentration in the plasma samples, the value for the endogenous level of urea was subtracted.

The urea space was calculated using the following equation:-

$$\text{Urea space} = \frac{\text{Wt of urea injected}}{\text{Concentration of urea at } T_o}$$

"T_o" refers to the time of injection, and the urea concentration at this point was calculated by back-extrapolation. Previous work (Bradbury, 1961) indicated that urea is metabolised during its dilution phase, and a steady state is never achieved. The extrapolation procedure accounts for the urea which is lost before complete dilution of the infused urea has been achieved.

(iv) Thiocyanate dilution

Sodium thiocyanate was used to measure extracellular space in the two sows in experiment 2. It was injected as a 10% solution, constituted from pure sodium thiocyanate and distilled water. The dose rate was 10 mg/kg liveweight. Prior to injection of the tracer, a blood sample was withdrawn from the animal, and used for calibrating the spectrophotometer.

In the first pig, blood samples were taken every 10 to 15 minutes up to 4 hours post-injection to determine the time of equilibration of thiocyanate in the body. Equilibration was found to occur within $1\frac{1}{2}$ to 2 hours of injection and thus in the second pig, blood samples were taken every 10 to 15 minutes for two hours.

The blood was collected in evacuated tubes which were inverted to allow the separation of the serum and the red cells. One ml of serum of each sample was treated in the manner described by Bowler (1944). Standard solutions of sodium thiocyanate were treated in a similar manner. The thiocyanate space was calculated using the following equation:-

$$\text{Thiocyanate space} = \frac{\text{Injected sodium thiocyanate (mg)} \times 100}{\text{mg sodium thiocyanate/100 ml serum}}$$

The value of the denominator was the serum thiocyanate concentration at equilibrium.

(v) Evans Blue dilution

In experiments 2 and 3, estimates of blood volume were made using Evans Blue in combination with haematocrit measurements.

A stock solution of Evans Blue was constituted prior to the beginning of each experiment. It was made as 1.5% w/v solution with sterile distilled water.

Immediately before the dye was injected into the pigs, 20 ml of blood were withdrawn through the catheter into an evacuated heparinised tube. About 15 ml of the blood was immediately centrifuged at 3000 r.p.m. for 15 minutes to separate the plasma from the red cells. The plasma was later used to make up a standard with a known concentration of Evans Blue, and also for adjusting the zero-setting of the spectrophotometer. Some of the remaining blood was introduced into a Wintrobe tube and centrifuged at 3000 r.p.m. for 30 minutes to determine the haematocrit value. Haematocrit readings were also made on the blood withdrawn 30 and 60 minutes after injection of the Evans Blue. No correction for the volume of trapped plasma was made.

About 1.8 g of the stock solution of Evans Blue was injected down the catheter by a syringe. The dose rate was 0.3 mg/kg liveweight. Immediately before and after injection, the syringe and needle were weighed

to the nearest 0.001 g. The Evans Blue solution was flushed down the catheter with 5 ml of heparinised saline solution after which the stylette was inserted.

Samples of blood were withdrawn through the catheter every 10 minutes during the hour following injection. They were centrifuged at 3000 r.p.m. for 30 minutes and the plasma was removed. The concentration of Evans Blue in the plasma samples was estimated from their optical densities measured at 620 nm on a Unicam SP600 spectrophotometer (Pye Unicam Ltd.). The concentration of dye in the plasma at zero time (T_o) was calculated by back extrapolation. The plasma volume was calculated from the following equation:-

$$\text{Plasma Volume} = \frac{\text{The weight of the dye injected (mg)}}{\text{Concentration of dye in plasma at } T_o \text{ (mg/100 ml)}}$$

The blood volume was calculated from the formula $(100 \times PV)/(100 - H)$ where PV = plasma volume, and H = the haematocrit value.

Precautions were taken in experiments 2 and 3 to avoid the possible effects of excitement on the state of the spleen. In experiment 2 the sow was confined in its cage for some days before the blood volume determinations were made so that it became accustomed to the surroundings, and also to the frequent manipulation of the catheters. In experiment 3, the Evans Blue determinations were made about five hours after the pigs had been confined in their cages. The reason for such a short interval of time was to permit the blood volume determinations to be made before any quantity of blood was withdrawn for the other dilution methods. The experimental procedure in experiments 2 and 3 was similar, and for each pig manipulations were made as quietly and as quickly as possible to minimise excitement in the animal.

(vi) Visual assessment

In experiment 3, when each pig weighed about 90 kg, its body

fatness was visually assessed by a panel of five judges. The panel judges were selected on the basis of their previous experience with pigs. The panel members were allowed to assess the fatness of each animal by any method, except of course, by direct measurement. None of the judges had any knowledge of the nutritional history of the pigs. Brief details of each judge are given below:-

Judge	A	A retail butcher
"	B	An experimental officer who has been in charge of pig experiments at the Institute for several years
"	C	A scientific officer whose work in the past has included the growth and development of farm animals
"	D	The Institute farm manager
"	E	A carcass dissection officer in the Meat and Livestock Commission

Each judge independently assessed the pigs into one of the following five gradings, each of which had a numerical value:- Very Fat = 1; Fat = 2; Average = 3; Lean = 4; Very Lean = 5. The range of this grading system was such that any pigs designated "Very Fat" were the fattest pigs each of the panel members have ever seen.

Before the first official meeting of the visual assessment panel (VAP), a number of pigs of varying degrees of fatness were visually assessed by each judge. The results obtained by each member were then openly discussed. The purpose of this was to accustom each judge to the type of pig which was going to be used, and to the grading system. However, during and between the official VAP meetings, no discussion was allowed and the results were not given to any of the members till the experiment was completed.

At each meeting of the VAP, two extra pigs of similar breed and weight were also assessed so that special scrutiny was not given to the

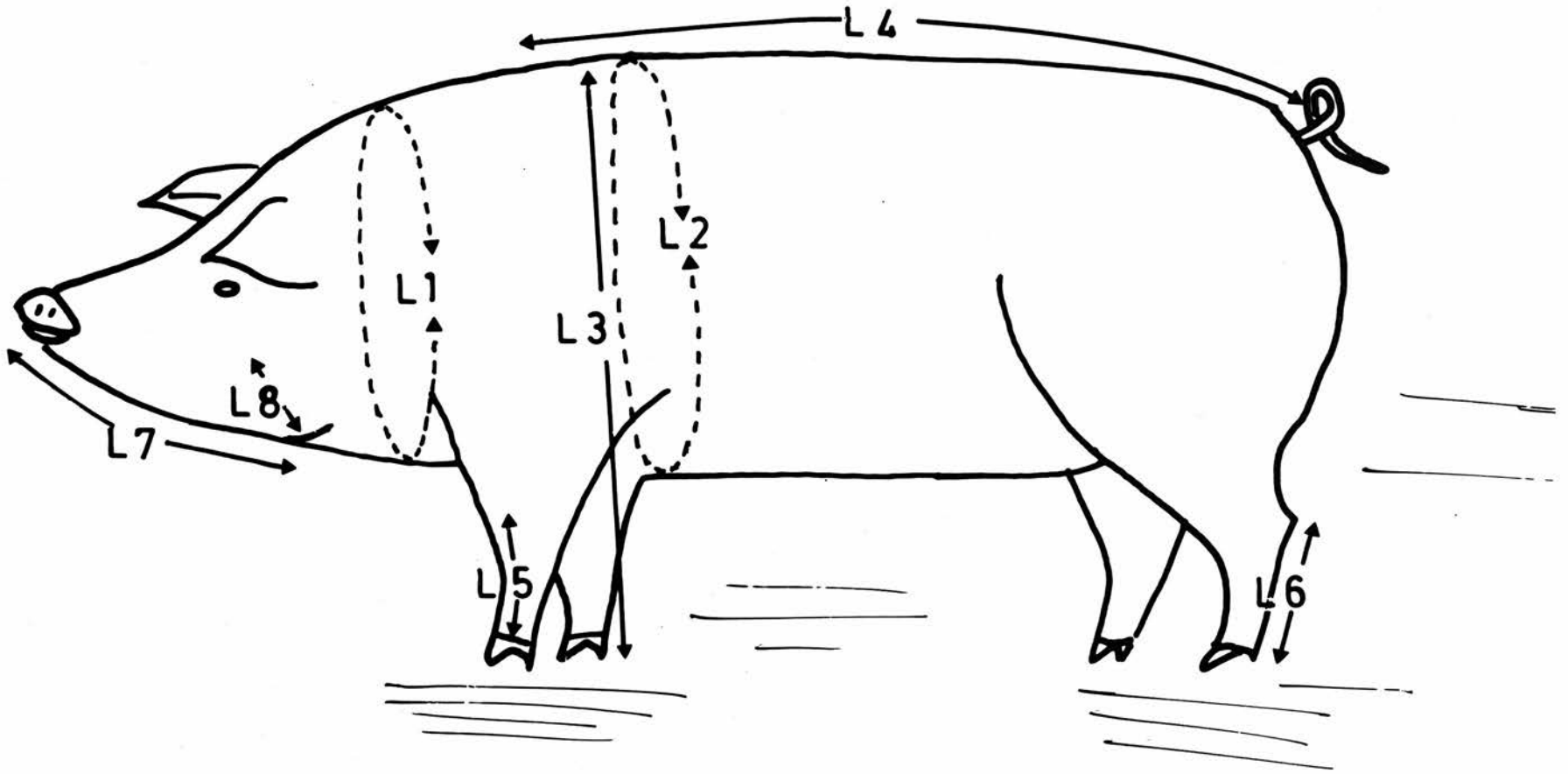


Fig. 6.2 Location of the External Measurements

experimental pig. The identification of the three pigs at each meeting was known only to the author.

(vii) External measurements

In experiment 3, several dimensions on the exterior of each animal and on the hanging carcass after slaughter were measured. The measurements made on the live animal were used for predictive purposes only, whereas those taken on the carcass were also used for evaluating the precision of the ultrasonic technique.

The measurements on the live animal were made when the pig weighed about 90 kg. Each pig was restrained in a cage and given a small quantity of feed in order to minimise its movement. The dimensions which were measured are shown in Fig. 6.2 and are described below. Each measurement was recorded twice when the animal was relatively motionless.

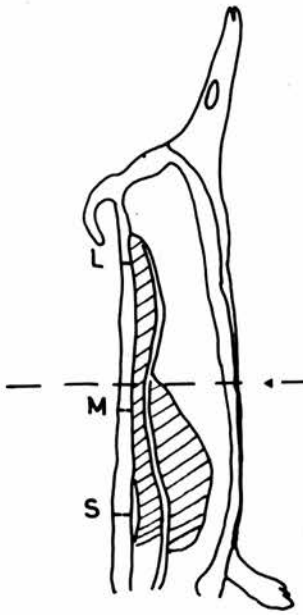
L1 The circumference of the neck was measured immediately behind the ears and lower mandible. The measurement was recorded whenever the head was in a horizontal plane.

L2 The heart girth was measured immediately behind the scapulae.

L3 The height at the shoulder was taken as the vertical distance between the ground and the top of the back opposite the scapulae when the animals' head was in a horizontal position.

L4 The length of the body from the shoulder to the tail was the distance following the contours of the back, from a point between the scapulae and the root of the tail. It was recorded whenever the backbone was in a horizontal plane.

L5 The length of the forearm was recorded as the distance from the tip of the elbow to the top of the claw.

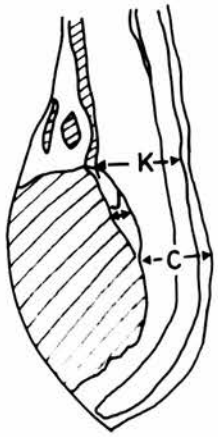


S Maximum depth of subcutaneous fat in the shoulder region

M Minimum depth of subcutaneous fat in the midback region

L Minimum depth of subcutaneous fat in the loin area

Fig. 6.3 Location of Dorsal Back Fat Measurements on the Hanging Carcass



K Depth of fat over the longissimus dorsi at 8cm from the mid line

C Depth of fat over the longissimus dorsi at 4.5cm from the mid-line

Fig. 6.4 Location of the C and K Back Fat Measurements on the Quartered Side

L6 The length of the hind leg was the distance from the tip of the elbow in the hind leg to the top of the claw.

L7 The distance from the front of the mouth to the posterior tip of the lower mandible was one of the quickest measurements taken !

L8 The width of the jaw was the distance from one side of the bottom jaw to the other, measured by calipers.

Measurements of backfat thickness (Fig. 6.3) were made on the side corresponding to that on which the ultrasonic measurements had been made. The carcasses were suspended vertically and the following dimensions were measured in duplicate by two independent operators:-

S - Maximum depth of subcutaneous fat in the shoulder region

M - Minimum depth of subcutaneous fat in the midback region

L - Minimum depth of subcutaneous fat in the loin area

The maximum deviation allowed between the four readings at each of the above-defined points was 2 mm. Repeat measurements were made if any reading was outwith this range.

Immediately after these measurements had been taken, the carcass side was quartered adjacent to the posterior side of the head of the last rib. On the fore-quarter, the C and K fat depths were measured at $4\frac{1}{2}$ cm and 8 cm, respectively, from the dorsal mid line (Fig. 6.4). The measurements were taken in duplicate by two independent operators. The maximum deviation allowed between the four measurements at each position was 1 mm.

(viii) Ultrasonic measurements

In experiment 3, measurements of backfat depth were made by the

ultrasonic technique, on the 24 pigs when they each weighed about 90 kg. Three operators each made the following series of measurements:-

U1 Maximum depth of shoulder fat

U2 Minimum depth of subcutaneous fat in the midback region

U3 Minimum depth of subcutaneous fat in the loin region

C The depth of fat over the longissimus dorsi - measured $4\frac{1}{2}$ cm from mid-dorsal line, opposite the head of the last rib

K The depth of fat over the longissimus dorsi - measured 8 cm from the mid-dorsal line, opposite the head of the last rib

The three operators were selected on the basis of their previous experience with ultrasonic appraisal of fat thickness in pigs. Operator A, a carcass dissection officer in the Meat and Livestock Commission, was a regular user of ultrasonic equipment and was considered highly experienced. Operator B had been on a course of instruction in the use of ultrasonic equipment on farm animals, and was considered a semi-skilled operator. Operator C, the author, had no previous experience with ultrasonic equipment and was completely inexperienced.

The ultrasonic equipment used was a Sonatest TE/6, which had an operating frequency of 2.5 mc/sec. The display was on a grid-type background which had a range of 0 to 100 mm.

To facilitate maximum contact between the skin and the probe, a commercial grade of liquid paraffin was poured on to the skin. In some of the hairy animals, the hair had to be removed before suitable contact with the skin could be made.

While the measurements were being made the pigs were restrained in a cage, and movement within the cage was minimised by allowing the pigs to eat a small quantity of feed.

On each pig the ultrasonic measurements were made in duplicate by

each operator, the recorded values being given in confidence to an independent scorer. The remaining two operators were not allowed in the room when the other was recording his results. Before the ultrasonic measurements were taken, each operator had to satisfy himself that the scale was accurately calibrated and that the positions for recording the C and K fat depths were correct.

Precautions were taken by each operator to ensure that (a) when the shoulder fat depth measurements were taken, the head of the pig was in a horizontal plane so that the subcutaneous fat over the shoulder was not distorted by stretching; (b) when the midback fat measurements were taken, the back of the pig was not arched, but in a horizontal plane.

(ix) Feed conversion ratio measurements

Measurements of feed conversion ratio (FCR) were made for each of the 24 experimental animals in experiment 3 over the growing period, 25 to 90 kg liveweight.

Definition of parameters used in FCR calculations. The growing period for each of the experimental pigs was defined as the number of days from when the pigs were first allowed access to the experimental diets X and Y, to the day prior to their confinement in the metabolism cages. The length of the growing period of each pig is shown in Appendix Table 3.

The amount of diet eaten during this period was calculated as the sum of the daily feed intakes, from the time of admission of each pig to the experiment, to the day prior to confinement to the metabolism cages. The total feed intake of each pig was corrected for feed refusals.

The liveweight gain of each pig was defined as the difference between the weight of the pig at the point of slaughter and the liveweight at the time of admission to the experiment. The weight at slaughter was considered the most appropriate weight of the animal, because the weights of the individual body constituents were calculated on this weight.

Calculation of the corrected feed conversion ratio. The corrected feed conversion ratio (CFCR) was defined as the feed intake - the feed equivalent of maintenance/liveweight gain. The calculation of CFCR for each pig involved determining the metabolisable energy (M.E. kcal) of the diet, and estimating the cumulative maintenance requirement during the growing period. The M.E. of each diet was calculated from the T.D.N. values of the individual constituents of the diet.

The TDN value of diet X was calculated to be 70.62% and that for diet Y, 69.18%. It was assumed that the digestible energy value (DE) of TDN was 4.4 kcal/g and that M.E. was approximately 95% of DE (Diggs, Becker, Terrill and Jensen, 1959). This gave values of 2952 kcal of M.E./kg for diet X and 2892 kcal of M.E./kg for diet Y.

The cumulative maintenance requirement of each pig during the growing period was calculated in the following manner:- The metabolic liveweight over the growing period was calculated. The values of liveweight (W) of each pig were transformed to the form $W^{0.56}$ and the mean metabolic liveweight was computed from, $[\frac{1}{2} W_1^{0.56} + \frac{1}{2}(W_1^{0.56} + W_2^{0.56}) \ldots \frac{1}{2}(W_n^{0.56} + W_n^{0.56}) \ldots + \frac{1}{2} W_n^{0.56}]/D$, where D was the number of liveweight recordings for each pig. The maintenance requirement was then calculated using Breirem's (1939) equation, $1963W^{0.56}$ x d, where d = the number of days in the growing period. The estimated maintenance requirements for each pig over the growing period are given in Appendix Table 3. The estimated energy requirement of each pig was subtracted from the total M.E. intake during the growing period. This provided a figure for the "energy available for production", which was then converted to kg of feed, by applying the M.E. concentration factor for each diet. The estimate of CFCR was made by dividing the feed available for production by the liveweight gain. The individual values are given in Appendix Table 3.

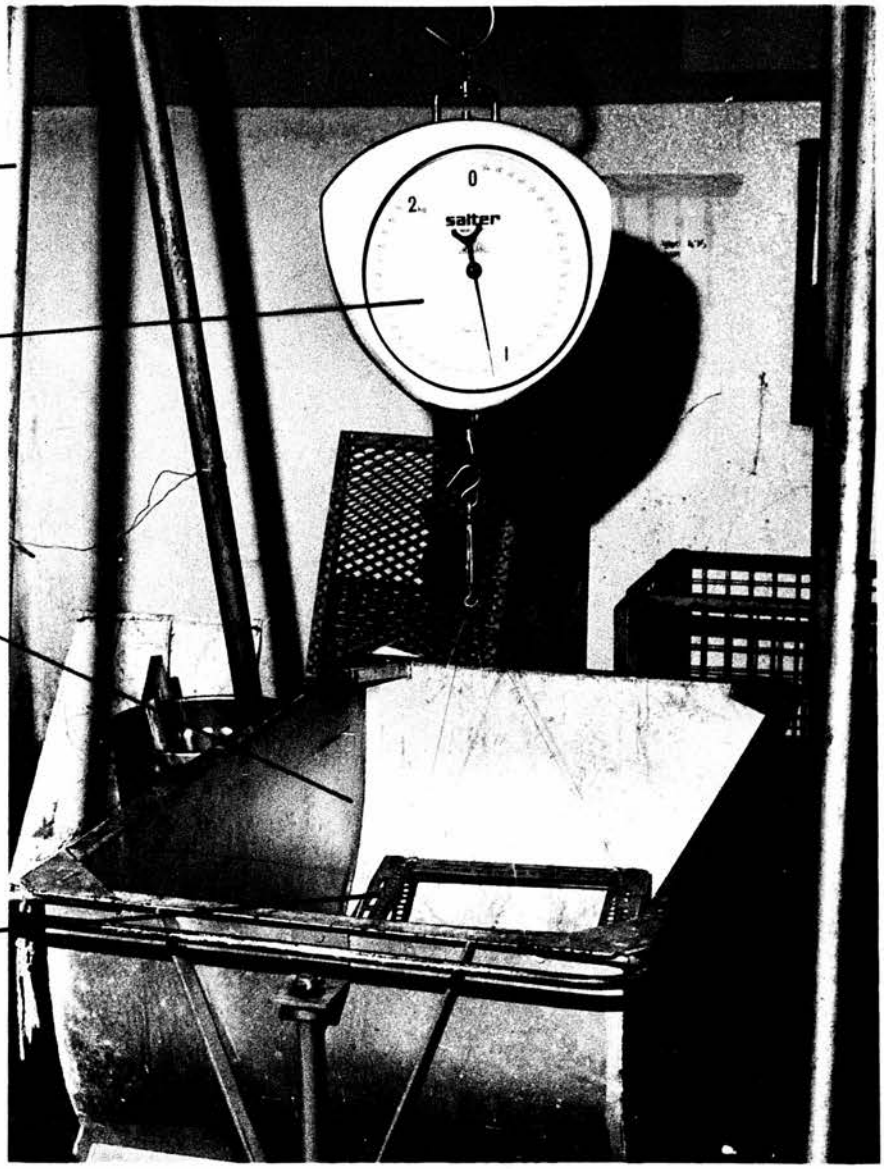


Fig. 6.5 Equipment for Measuring the Weight of the Half Carcass in Water

(x) Specific gravity measurements

In experiment 3, specific gravity measurements were made on the de-haired side of the carcass. The fore and hind quarters of the left side were weighed separately in air to the nearest 50 g and were then each placed on an aluminium tray in a bath (120 cm x 75 cm x 55 cm) of cold water and re-weighed to the nearest 5 g (Fig. 6.5).

The weighings in water were made in duplicate, and were recorded immediately the pointer on the balance became stationary.

The calculation of the specific gravity for the whole side was made from the following equation:-

$$\text{Specific gravity of the carcass side} = \frac{\text{Wt of fore and hind quarters in air}}{\text{Wt of fore and hind quarters in air} - \text{Wt of fore and hind quarters in water}}$$

The temperature of the water in which the carcass quarters were weighed was also noted.

CHAPTER 7

THE APPLICATION OF THE POTASSIUM 42 AND DEUTERIUM OXIDE DILUTION TECHNIQUES TO THE BACON PIG — EXPERIMENT 1

Introduction

In this chapter the results of experiment 1 are given in which the ^{42}K and the D_2O dilution techniques were applied to bacon pigs. The analytical procedures for both of these techniques have been described in Chapter 6.

Design of the experiment

Details of the animals used, their management and housing during the growing period have been described in Chapter 6. Twenty castrated male pigs were reared along different growth curves from about 25 to about 90 kg live-weight. The mean growth rates and the percentage of lipid in the empty bodies of the pigs on each of the treatments are shown in Table 7.1.

The dietary treatments which are also described in Table 7.1 were designed to promote large differences in body fatness. They were combinations of increasing dietary protein concentration and decreasing feed intake. The composition of the diets used is shown in Appendix Table 4.

Table 7.1

Mean growth rates (kg/d) of pigs given different rations, and in parentheses, mean percentages of lipid in their ingesta-free carcasses

Daily feed intake ($g/kg^{0.73}$)	Diet 1 10% protein	Diet 2 14% protein	Diet 3 18% protein
70	-	-	0.31 (18)
80	-	-	0.39 (23)
90	-	0.48 (27)	-
100	0.50 (31)	-	0.59 (25)
110	-	0.63 (26)	-
120	0.59 (33)	-	-
130	0.65 (35)	-	-

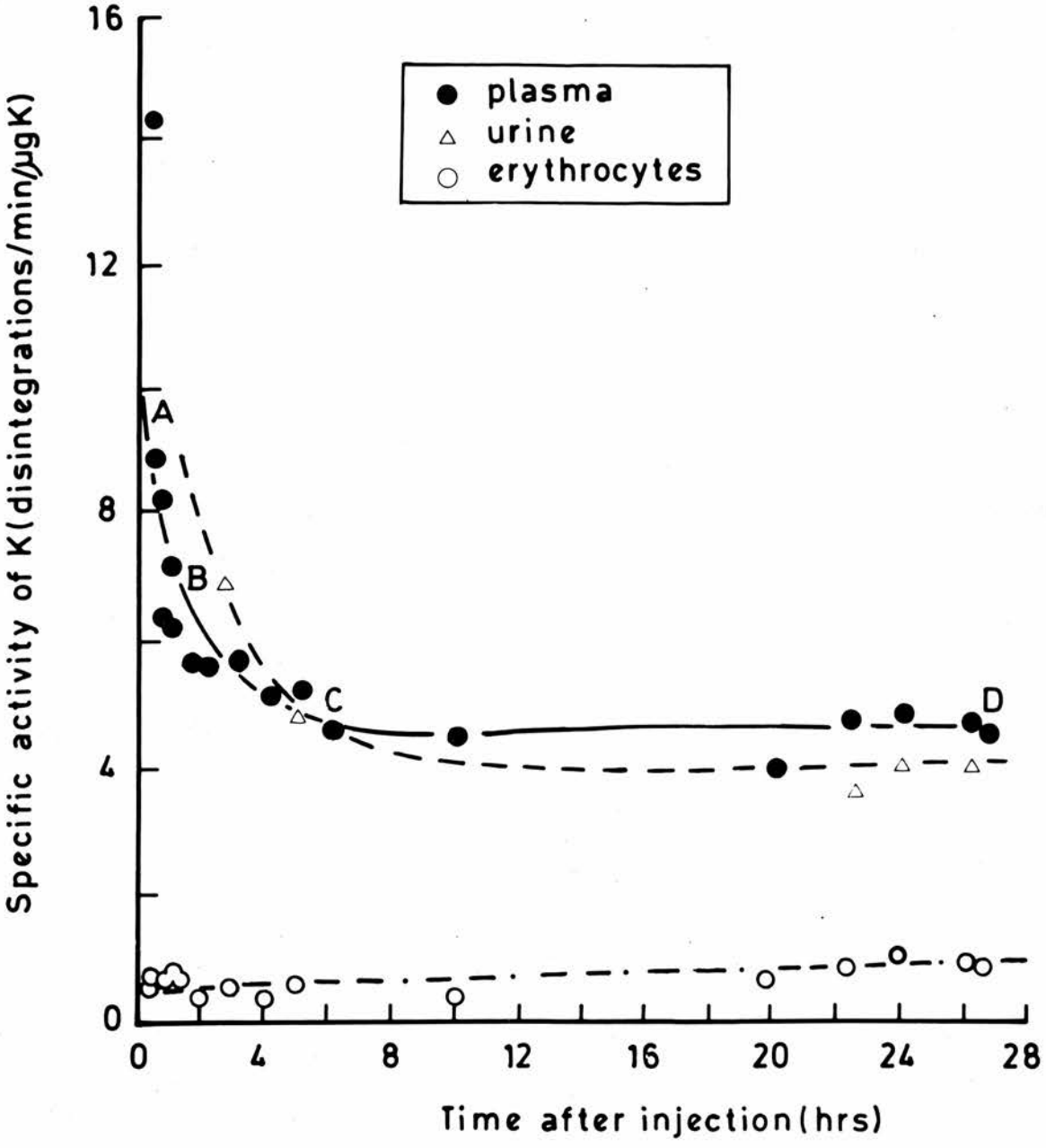


Fig. 7.1 Time Course of Specific Activity of Potassium in Plasma, Urine and Erythrocytes of Pigs after the Intravenous Injection of ^{42}K.

At about 90 kg liveweight, each pig was removed from its pen and put in a metabolism cage.

Results

(a) The potassium 42 dilution technique

The following results apply to 17 of the 20 pigs which were injected with ^{42}K. For two pigs final urine samples were not voided, and for the other pig, only two urine samples were obtained which varied widely in their specific activity.

(i) The time course of the specific activity of the plasma, red cells and urine. In the first few animals the time-course of the specific activity of the plasma, red cells and urine was monitored for 24 hours after the injection of the ^{42}K. The decline in the specific activity of each of these components is shown in Fig. 7.1.

It can be seen that the equilibration of the label with the body potassium was virtually complete within 10 to 12 hours. The plasma specific activity curve was very similar to that obtained by Fenn, Noonan, Mullins and Haege (1941) in rats. Three phases of the curve can be distinguished, which reflect the exchange of the label with the body potassium. In the first phase there was a rapid reduction in the plasma specific activity soon after injection of the label (portion A-B of the curve) which possibly reflects the rapid disappearance of the label from the blood-stream into the body. This was followed by a more gradual decline in the plasma specific activity (portion B-C of the curve), possibly indicating a relatively rapid clearance of the label into the "rapidly exchanging" organs and tissues. The third phase (portion C-D of the

graph) was characterised by a slow decline in the plasma specific activity, which suggests that most of the label had exchanged with the body potassium, and that the label was being slowly lost from the body.

The specific activity curve of the red cells is characteristic of a body compartment with an extremely slow turnover rate (Fenn et al., 1941; Walker and Wilde, 1952). From Fig. 7.1 it can be seen that at 24 hours the specific activity of the red cells was quite different to the plasma specific activity value.

The urine specific activity curve was similar to that of the plasma, except that the decline in specific activity was not as pronounced.

(ii) Loss of the label from the body. For the purpose of calculating the total exchangeable potassium of the empty body, the loss of the label into the faeces, gut contents and urine during the equilibration period, was calculated for each pig (Appendix Table 5). It was found that, of the total activity injected into each of the pigs, on average 0.3 $\pm$ 0.45% was excreted into the faeces in 22 to 28 hours. This was the average value for 17 pigs, although it included the results from 9 pigs which voided no faeces during the equilibration period. The mean value for the remaining 11 animals was 0.43%. The loss of label into the gut contents during the same period amounted to 1.3 $\pm$ 1.07% of the injected dose. The main loss of label was in the urine in which 3.3 $\pm$ 1.78% of the injected label was excreted in 22 to 28 hours.

The mean total loss of the label from the body during the 22 to 28 hours following injection amounted to 4.9 $\pm$ 1.98% of the total label injected.

(iii) Calculation of the total exchangeable potassium. The total exchangeable potassium (K_e) of each pig was calculated from the specific activity (SA_e) of the urine or plasma at about 22 to 28 hours post-injection.

The calculation was as follows:-

$$K_e = \frac{I - L}{SA_e}$$

where I = the total activity injected

L = the total activity lost in the urine, faeces and gut contents

K_e = total exchangeable potassium calculated either from the plasma specific activity or from the urine specific activity.

The total exchangeable potassium of each pig, calculated from the plasma specific activity (K_{ep}) and from the urine specific activity (K_{eu}) was compared with the chemically-determined potassium (K_c) of the carcass. The individual values of K_{ep}, K_{eu} and K_c are shown in Appendix Table 6, the mean values being 155.9 g, 161.6 g and 163.6 g, respectively.

The regression equations relating the estimates of exchangeable potassium to the chemically-determined potassium for the 17 pigs are given:-

$$K_c = 35.49 + 0.778\ K_{eu} \quad RSD = \pm 7.81\ g\ K_c \quad r = 0.865$$

$$K_c = 126.07 + 0.241\ K_{ep} \quad RSD = \pm 17.28\ g\ K_c \quad r = 0.399$$

From a comparison of these two equations, it is clear that the estimate of the body potassium made from the urine specific activity value was superior to that made from the plasma specific activity value. The total exchangeable potassium calculated from the urine specific activity was therefore used in further calculations.

(iv) <u>The relationships between K_{eu} and various components of the body</u>. The objective of the study was to predict the amounts of certain body components in the live animal, from the exchangeable potassium. These components were body water, total body lipid, fat-free body mass, protein and fat-free dry matter. The values of these components for each of the 17 pigs are given in Appendix Table 7 and their relationships with K_{eu} are given in Table 7.2.

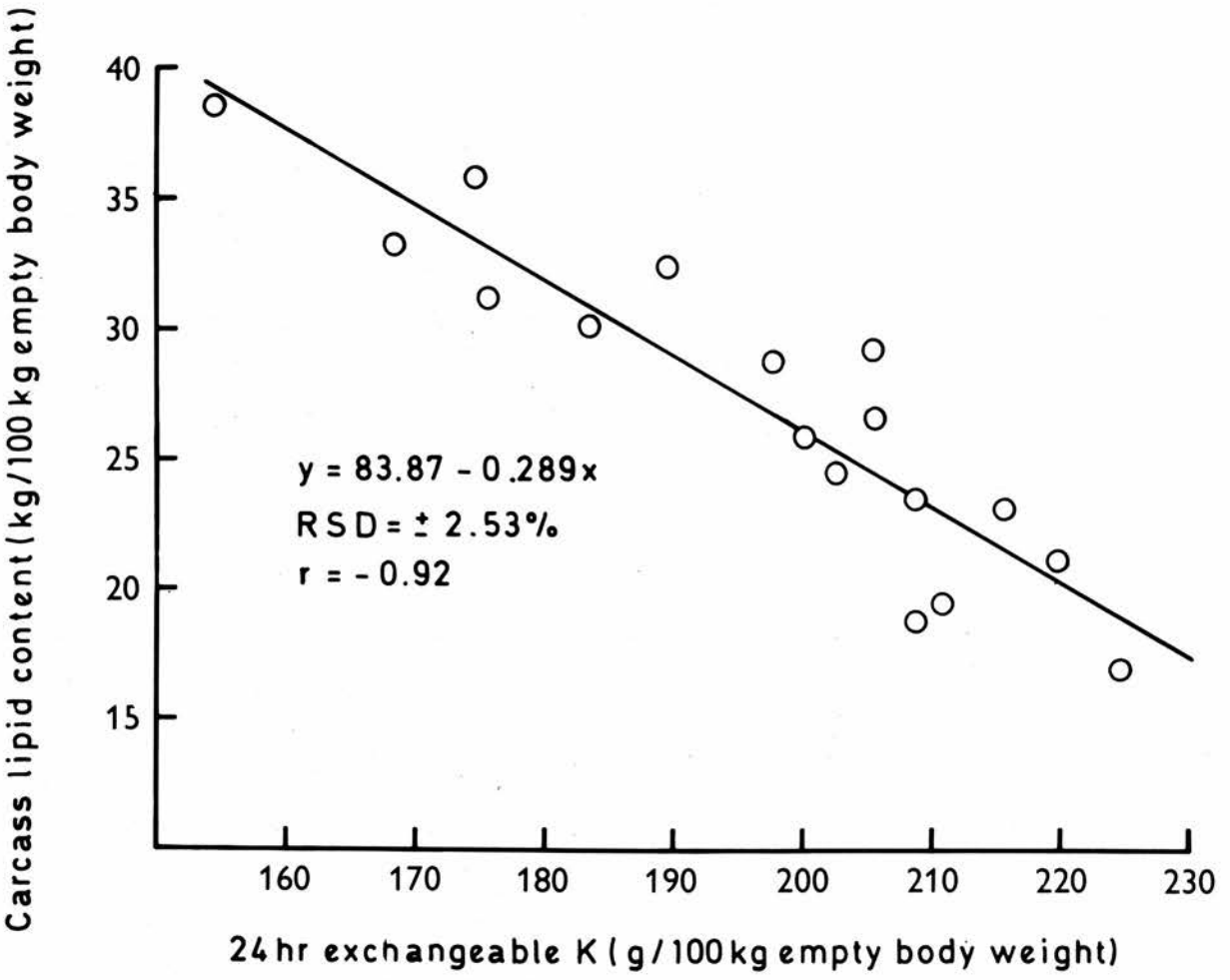


Fig. 7.2 The Relationship Between the Percentage Lipid in the Ingesta-Free Body and the 24 Hour Exchangeable K Calculated from Specific Activity Expressed per 100 kg Ingesta-Free Body Weight.

Table 7.2

Regressions of the weights of various body components of pigs on the 24 h exchangeable potassium (g) predicted from urine specific activity (K_{eu})

Dependent variables (y)	Units	Regression equation	RSD	r
Fat-free mass	kg	$y = 16.6 + 0.270\ K_{eu}$	1.80	0.93
Fat-free dry matter	kg	$y = 4.27 + 0.0699\ K_{eu}$	0.79	0.84
Total protein	kg	$y = 5.07 + 0.048\ K_{eu}$	0.72	0.76
Total water	kg	$y = 12.3 + 0.200\ K_{eu}$	1.99	0.87
% lipid in EB		$y = 83.9 - 0.289\ K_{eu}/EB$	2.5%	0.92

For the prediction of body lipid from K_{eu}, both the dependent and independent variables were expressed as a percentage of the empty body-weights. This was to eliminate the variation in liveweight which otherwise may have confounded the inverse relationship between the proportions of fat and lean in the body. The relationship is also shown in Fig. 7.2.

The corresponding relationships between the various body components and the chemically determined potassium of the body (K_c) are shown in Table 7.3.

Table 7.3

Regressions of the weights of various body components on the total potassium (g) determined by chemical analysis of the carcass

Dependent variables (y)	Units	Regression equation	RSD	r
Fat-free weight	kg	$y = 17.7 + 0.264\ K_c$	2.84	0.82
Fat-free dry matter	kg	$y = 6.5 + 0.092\ K_c$	0.14	0.99
Total protein	kg	$y = 2.2 + 0.066\ K_c$	0.39	0.94
Total water	kg	$y = 17.1 + 0.17\ K_c$	2.95	0.67

(v) Discussion. The purpose of this experiment was to predict the composition of the live pig from the exchangeable potassium (K_e). This was calculated from both the plasma and urine equilibrium specific activities. K_e calculated from the urine specific activity was highly correlated with the chemically-determined potassium of the carcass. Most surprising was that although successive plasma specific activity values were similar after equilibrium, the urine values were found to give the most accurate estimates of total body potassium. Indeed, the plasma and urine specific activity curves were similar, but in many cases the curves diverged at about the point of equilibrium. It is very difficult to ascribe any physiological cause for this and it is suggested that errors in the analytical and counting techniques may have been responsible for the discrepancy in the equilibrium values. In addition, the infrequency of the urine samples made it difficult to establish smooth urine specific activity curves.

The findings obtained in this experiment are similar to those of Flear, Cooke, Sivyer and Domonet (1963). These workers reported that successive values of the urine specific activity made 24 hours after injection, varied considerably in human subjects. They concluded that equilibration of the label with the body potassium was achieved at different times in different subjects. O'Toole, Cech and Peterson (1967) suggested that the extent of body fatness and starvation also affected the rate of exchange between the label and the body potassium. In this experiment the animals were starved of food and water before and after the injection of ^{42}K. This may account for some of the variability between the urine and plasma estimates of K_e, although inspection of the data did not reveal any association between the duration of starvation or body fatness and the divergence between the two specific activity curves.

A comparison of the relationships between K_{eu} and K_c and the chemically-determined constituents of the body indicated that although K_c formed a relatively constant fraction of the fat-free dry matter, it was not as closely associated with the fat-free body mass. Conversely, K_{eu} was more closely associated to the fat-free body mass than to the fat-free dry matter. This suggests that the potassium/water relationships in the body were not constant. Indeed the correlation between K_c and the empty body water was only 0.67. These results suggest that the potassium associated with the body water was more readily exchangeable than that associated with tissues containing little water.

On analysis of the chemical data of the pigs, a precise relationship between the amount of potassium and protein (N x 6.25) in the empty bodies of these pigs was found. The mean K:N ratio (mg/g) was 78.3 which agrees closely to values reported by other workers for pigs (Table 7.4). The coefficient of variation of this value was 3.38%.

Table 7.4

Estimates, from the present work and from the literature of the proportion of K in the fat-free body (K/FFW) and of the ratio K:N in the bodies of pigs of 90 kg liveweight

Source of Values	K/F.F.W. (g/kg) Mean	S.D.	K:N (mg/g) Mean	S.D.	n
Oslage (1965)	2.58	± 0.100	76.2	± 3.08	6
Pfau (1966)	2.77	± 0.050	78.7	± 4.00	4
Stant, Martin and Kessler (1969)*	2.71	± 0.089	80.2	± 3.07	5
Present results	2.68	± 0.135	78.3	± 2.65	17

*Eviscerated hairless carcass

(vi) Appraisal of the method and conclusions. The ^{42}K dilution technique was found to be extremely accurate in predicting body composition in the live pig. The method warrants further investigation and application, especially in less artificial conditions in which extremes of body fatness are not common. For more practical applications it may be desirable to investigate a) the use of the cheaper forms of ^{42}K which are available, b) the oral introduction of the label into the body, c) the counting of the γ disintegrations from ^{42}K which could save labour in the preparation of the body fluid samples for counting, and d) the accuracy of spot urine or blood samples in the determination of exchangeable potassium.

(b) The deuterium oxide dilution technique

On ten of the pigs which were used for the appraisal of the ^{42}K dilution technique, estimates of total body water were made using D_2O. The calculation of the body water space of each pig was made using the equilibrium plasma D_2O concentration. In those pigs in which samples of blood and urine were obtained up to 24 hours post-injection, it was possible to follow the time-course of equilibration within the body. Equilibration of the D_2O within the body was found to be virtually complete within 2 to 3 hours, which is similar to the equilibration time for tritium as found by Kay (1963).

(i) Loss of the label from the body. Homogenous samples of the gut contents and faeces were treated by the Dean and Stark method to yield water/D_2O mixtures which were then purified by the vacuum distillation method. The concentrations of D_2O found in the faeces and gut contents of three pigs are shown in Table 7.5 and are compared with the plasma D_2O concentrations.

Table 7.5

Concentration of deuterium oxide in faeces, gut contents and plasma at equilibrium

Pig No.	Plasma D_2O (%)	Faeces D_2O (%)	Gut contents D_2O (%)	Proportion of label in faeces and gut contents (%)
8	0.184	-	0.179	3.40
9	0.186	-	0.180	3.24
10	0.176	0.172	0.175	4.24

The concentration of D_2O in urine was difficult to determine by conventional methods. Certain compounds were present which rapidly attacked the barium fluoride windows of the Infra Red Spectrophotometer cells. For this reason, the urine samples were not analysed for D_2O.

(ii) Comparison of the D_2O space estimates with the total body water and the empty body water. The D_2O space was compared with both the total body water (TBW) and the empty body water (EBW) obtained by desiccation in each of the ten pigs (Table 7.6).

Table 7.6

Comparisons between the deuterium oxide space (kg) and total body water (kg) and empty body water (kg) in ten of the pigs in experiment 1

Pig No.	Total body water	Empty body water	D_2O
1	50.10	47.77	56.39
2	49.44	47.15	55.71
3	49.20	45.39	53.28
4	47.96	44.00	50.20
5	44.64	42.83	48.82
6	46.17	44.08	50.76
7	42.33	40.82	47.90
8	42.45	40.59	43.79
9	43.92	41.37	44.87
10	51.25	48.02	53.21
Means and standard deviations	46.75 $\pm$ 3.114 kg	44.20 $\pm$ 2.681 kg	50.49 $\pm$ 4.042 kg

The results shown in Table 7.6 indicate that deuterium oxide overestimated both the total body water and the empty body water. The mean difference between the total body water space and the D_2O space is 3.75 kg and the Standard Error (S.E.) of this value is $\pm$ 0.635 kg. The mean difference between the empty body water space and D_2O space is 6.29 kg and the S.E. of this value is $\pm$ 0.601 kg.

These data indicate that the D_2O may have exchanged with non-aqueous hydrogen in the body or it may have become excessively localised in some fluid compartment. There is also a possibility that a positive contamination of the plasma samples during the analytical procedure, may have occurred.

Analysis of these possible sources of error for their relative contribution is difficult because most of the pigs were blood-sampled and slaughtered before the analysis for D_2O had been completed. It is tempting, however, to draw some conclusions from the data which are available. For instance, on closer inspection of Table 7.5 it can be concluded that D_2O does not become excessively localised in the gut contents. From Table 7.6 it can be seen that the D_2O space was very similar to the total body water space in pigs 8, 9 and 10. The mean D_2O space of these pigs was 47.29 $\pm$ 4.209 kg compared with a mean total body water value of 45.76 $\pm$ 3.848 kg. These results indicate that D_2O overestimated TBW on average by 3.53%, a figure which is of the same order as those obtained by Schloerb *et al.* (1950) and Hevesy and Jacobsen (1940). These workers have suggested that D_2O combines with the labile hydrogen ions of the protein and fat in the body which have a water equivalent amounting to 0.5 to 2% of the body weight.

It is also relevant to note that in pigs 8, 9 and 10, the plasma samples were processed and analysed immediately after being withdrawn from

the body, and were not stored in a freezing unit as were the plasma samples from the first seven pigs. This may have reduced the possibility of moisture contamination of the samples (which would tend to result in high body water values) during storage. In addition the double vacuum distillation technique was discontinued after analysis of the plasma samples of the first seven pigs, in favour of single vacuum distillation. This was because the double vacuum distillation technique was slow and laborious and the yields of water/D_2O mixtures were often very low. The adoption of the single vacuum distillation technique may have reduced the possibility of moisture contamination of the samples during the analytical procedure. Unfortunately the two methods were not compared for their precision.

Despite the fact that there were obvious differences in the precision of the D_2O technique in this experiment, the results which were obtained for the 10 pigs were treated as one group. The relationships are shown in Table 7.7.

Table 7.7

The relationships between empty body water, total body water, and the D_2O space in ten pigs

Dependent variables (y)	Units	Regression equation	R.S.D.	r
Total body water	kg	$y = 0.686.D_2O + 12.11$	1.59	0.89
Empty body water	kg	$y = 0.620.D_2O + 12.88$	1.06	0.94

These equations indicate that despite the fact that there were often large discrepancies between the estimated body water and actual body water as shown in Table 7.6, D_2O gave fairly accurate estimates of the amounts of water in the empty body and in the whole body. The R.S.D's of the equations are relatively small in comparison to the mean values and represent 3.40 and 2.40%, respectively, of the total body water and empty body water values.

Regression equations were also computed for the estimation of other body components by D_2O dilution. These are shown in Table 7.8.

Table 7.8

The prediction of fat-free weight (kg), protein (kg) and the percent body lipid from the D_2O space in ten pigs

Dependent variables (y)	Units	Regression equation	R.S.D.	r
Fat-free mass	(kg)	$y = 0.836\ D_2O + 17.35$	1.27	0.95
Total protein	(kg)	$y = 0.174\ D_2O + 3.99$	0.76	0.72
Percent lipid in the empty body		$y = 87.06 - 1.99\%\ D_2O$	2.14%	0.89

It can be seen that the weights of fat-free tissue and protein in the empty body were predicted with a fairly high degree of accuracy, the R.S.D's of the respective equations being 5.9% and 2.1% of the mean values. D_2O dilution also gave a fairly accurate estimate of the proportion of lipid in the empty body (R.S.D. = $\pm$ 2.14% lipid). In this equation, both variables were expressed as a percentage of the body weight to allow for the small variations in body weight which would otherwise tend to reduce the closeness of the inverse relationship between the proportions of lean tissue and fat in the body. D_2O was expressed as a percentage of the total body weight because it is possibly the most meaningful ratio in practical terms, but lipid was expressed as a percentage of the empty body weight. Some of the variability in the relationship is possibly attributable to the variable amount of water in the gut contents and bladder.

(iii) Discussion. Deuterium oxide was found to equilibrate in the body in 2 to 3 hours and analyses of the faeces and gut contents indicated that D_2O rapidly crossed the gut-wall.

The values obtained for the D_2O space for the first seven pigs did not promote much enthusiasm for the method, and several sources of error

which may have contributed to such variable values have already been discussed in the text. It is obvious, however, that in future studies in which D_2O is to be used, that the positive or negative moisture contamination of the plasma samples during the analytical procedure should be prevented as far as is practical. Calculations show that in pig 1 for example (see Table 7.6), assuming that no other factors are involved, it would require only 0.6 ml of water to contaminate a 5 ml plasma sample to cause a 6 kg difference between the estimated and actual total body water space.

The results obtained for the last three pigs indicate that D_2O dilution can give close estimates of total body water, although it would be dangerous to draw too many conclusions from only three data points which gave the "best" results. However, the fact that the analytical procedure for D_2O was simplified for these three pigs and that it was performed immediately after the samples had been withdrawn from the body suggests that the D_2O method has a potential use for estimating body composition in the live pig, when it is performed under controlled conditions. Indeed, the regression equations computed for the ten pigs show that despite the several factors which were possibly involved, a fairly accurate prediction of body composition can be made, using D_2O.

(iv) Appraisal of the method and conclusions. The D_2O dilution technique was found to give excessive values for body water in the ten pigs in which it was applied. From the data obtained on three pigs it was found that the D_2O crossed the gut and equilibrated with the water in the intestinal tract. There was also an indication from three of the pigs that D_2O may have exchanged with some of the non-aqueous hydrogen in the body, but there are no analyses to confirm or refute this suggestion.

For future work it would be advantageous to (a) minimise the quantity of urine in the bladder and the amount of intestinal contents

(b) analyse the plasma samples soon after withdrawal from the body, and to ensure that no positive or negative moisture contamination of the samples occurred during the analytical procedure.

In general, the results obtained in these ten pigs indicate that D_2O dilution can give fairly accurate estimates of body composition and thus the method warrants further investigation.

Summary

a) ^{42}K injected into 17 bacon pigs equilibrated with the body potassium within 10 to 12 hours. The loss of label from the body in 22 to 28 hours was, on average, $4.9 \pm 1.98\%$ of the total label injected, the main loss being in the urine. The total exchangeable potassium calculated from the urine equilibrium specific activity agreed closely with the chemically determined potassium of the carcass. The total exchangeable potassium (K_{eu}) was highly correlated with the fat-free body weight and the percentage of lipid in the empty body.

The technique was considered to be sufficiently accurate for the measurement of *in vivo* body composition to warrant further investigation.

b) D_2O injected into 10 bacon pigs equilibrated with the total body water in about 2 to 3 hours. The loss of label into the faeces and gut contents, in this time, amounted to about 3-4% of the label injected. Only small concentrations of D_2O were recorded in the urine, but difficulties were encountered in the analysis of urine. Comparisons of the D_2O space with the empty body water and total body water proved to be disappointing in most of the pigs. Suggestions were proposed as to the likely causes of these discrepancies, although there was little experimental evidence to confirm or refute these proposals. In each of three pigs in which the plasma samples had been analysed immediately after withdrawal from the body by a simpler vacuum distillation procedure, the D_2O space was found

to be very similar to the total body water space.

It was found that the D_2O space was fairly accurate in estimating both the total body water, empty body water and other body components, and therefore the method was considered suitable for further investigation.

CHAPTER 8

AN EXPLORATORY INVESTIGATION INTO THE APPLICATION OF UREA, SODIUM THIOCYANATE AND EVANS BLUE IN THE LIVE PIG - EXPERIMENT 2

Introduction

The objective of this investigation was to acquaint the author with the analytical procedure of certain indirect methods and to apply them singly, and in combination with each other in the pig and to assess their suitability for inclusion into the second part of the study. They were dilution techniques which have been used for measuring body water (urea), extracellular space (sodium thiocyanate) and blood volume (Evans Blue) in several species including the pig but have rarely been used for predicting body composition. D_2O was again used for the purpose of comparing the estimates of body water with those obtained from the urea dilution method.

Design of the experiment

The exploratory nature of this experiment did not necessitate the rearing of animals specifically to test these methods. The application of the methods to mature sows was considered satisfactory to provide the necessary information. Two barren sows which were due to be dispatched to the local bacon factory were used.

Preliminary investigations into the analytical procedures of each of the three methods were made in the laboratory. The purpose of this was to acquaint the author with the techniques involved, and also to determine if there were any interference effects in the analysis of one tracer when other tracers were also present.

Following this, the methods were applied simultaneously to the two

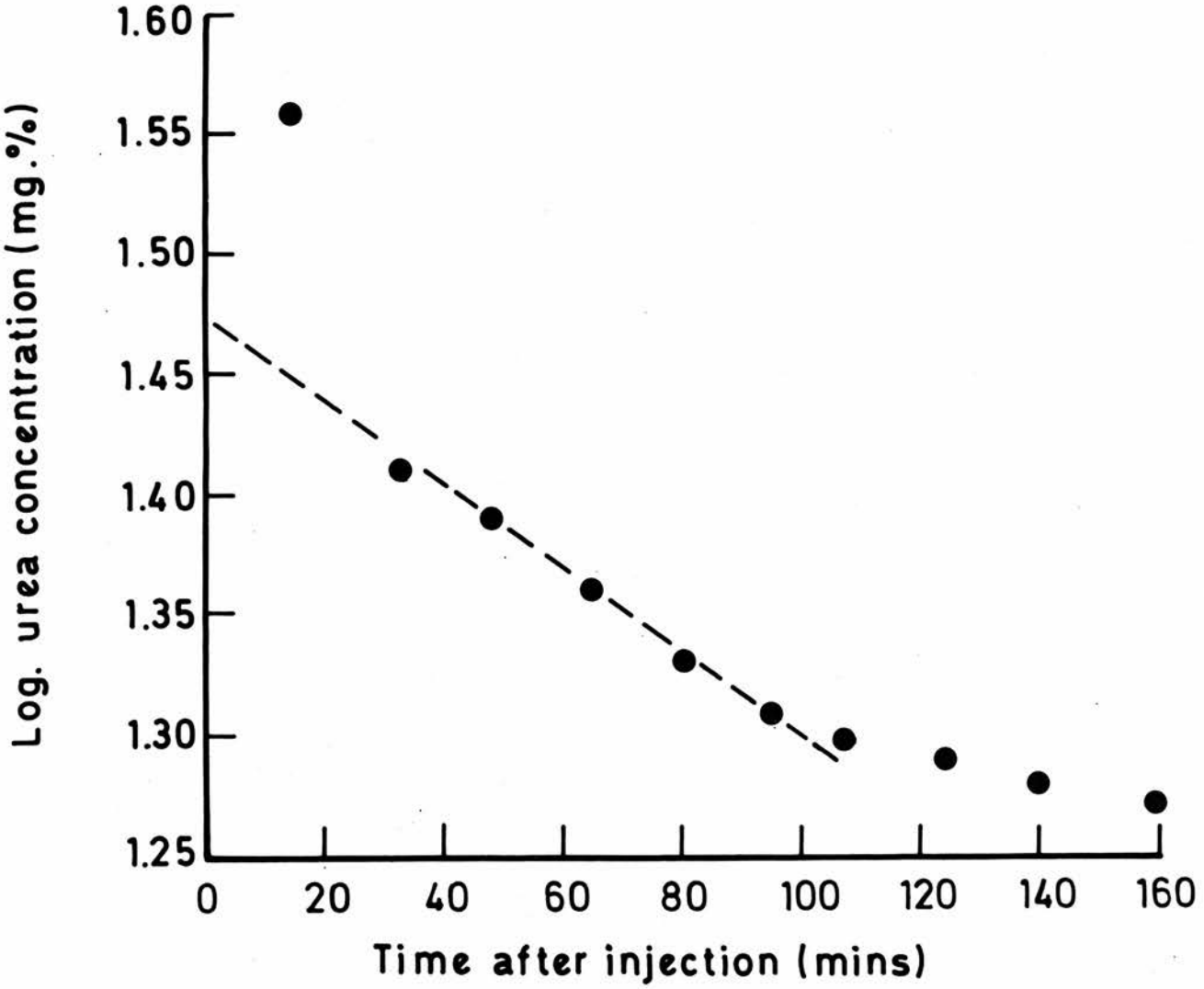


Fig. 8. 1. Urea Dilution Curve Showing The Back Extrapolation to T_o

sows. An attempt was made to measure the repeatability of each method.

Results

(a) The urea dilution technique

(i) Laboratory methodology. The technique was easy to implement, and the automated analytical technique saved time and labour in the estimation of urea in the plasma samples. The concentration of urea (mg/100 ml) in the plasma samples was determined by comparison of the optical densities of the plasma samples with the optical densities of a range of standard urea solutions, treated in the same manner. A linear response between the optical density and the concentration of urea was found in the concentration range 0 to 100 mg/100 ml of urea. A preliminary test was also implemented to determine the recovery of urea from plasma solutions. From 10 plasma solutions, in which the concentration of urea ranged from 10 mg/100 ml to 100 mg/100 ml, the mean recovery was calculated to be 98.50% (S.D. = $\pm$ 1.071%). No interference in the estimation of urea was found from D_2O thiocyanate or Evans Blue. The peak optical densities of thiocyanate and Evans Blue were measured at 470 nm and 620 nm, respectively, compared to the 530 nm for the peak optical density of urea.

(ii) Application of the method to sows. After its introduction into the blood-stream, urea is metabolised fairly quickly, and a back-extrapolation procedure is required to determine the concentration at zero time (T_o). To facilitate this calculation, the logarithm of the concentration of the urea in the plasma samples, corrected for the initial endogenous level of urea, was plotted against time (Fig. 8.1). It can be seen that there was a marked variation in the slope of the curve with time which suggests that there was either a variable endogenous production of urea during the course of dilution of the tracer, or that the exogenous urea was metabolised at different rates, as its dilution in the body progressed. Obviously any

variation in the slope of the curve causes great variation in the intercept value (log. urea concentration). In Fig. 8.1 for the first sow, the intercept was calculated to be 28.51 ml/100 ml, which gave a figure of 125.8 litres for the total body water. The estimate of the D_2O space in the same pig was 109.6 litres which suggests that the total body water space was considerably overestimated by urea. However, caution must be applied in the interpretation of these data, in which the results of one indirect method are used to validate another indirect method.

In the second sow, repeat estimates of the urea space were made on three successive days, and compared with the D_2O space. A similar variation in the slope of the dilution curve was observed, even over a shorter period of sampling. The estimates of TBW made by urea and D_2O are shown in Table 8.1.

Table 8.1

Comparison of the estimates of total body water by urea and deuterium oxide in one sow

	Urea space (litres)	D_2O space (litres)
Day 1	92.70	75.38
2	71.69	73.49
3	85.76	73.06
Mean	83.38	73.98
Standard Deviation	± 8.740	± 1.008

Further estimates of TBW, using this sow had to be discontinued because the sow developed a high temperature on the fourth day of the experiment.

(iii) Discussion. In the interpretation of the results of Table 8.1, TBW would not be expected to remain constant over a period of three days, especially if the pig was being subjected to periods of starvation

during this time. On the other hand, the estimates of TBW by urea indicated that wide variations occurred in the TBW from day to day. For example, between Day 1 and Day 2 there was a loss of TBW of 21.01 litres. These results can be compared with the D_2O estimates of TBW which showed only a small variation from day to day and did not indicate that wide fluctuations in the TBW space occurred. The results of this experiment, although limited, suggest that the use of urea in the estimation of TBW space could lead to considerable inaccuracies in the estimation of body composition.

(iv) Appraisal of the method and conclusions. The urea dilution technique was simple to implement and was much less expensive than the D_2O technique. Application of the method to two sows showed that it was not highly repeatable, there being large daily variations in the urea space. It is possible that variability in the endogenous production of urea or in the metabolism of the injected urea, are the main factors responsible for this large daily variation in urea space. The method was not considered sufficiently reliable to be included in further investigations.

(b) The thiocyanate dilution technique

(i) Laboratory methodology. Preliminary investigations were designed to test the linearity of response of colour intensity with increasing thiocyanate concentration, and to determine the recovery rates of thiocyanate from serum samples over a wide range of thiocyanate concentrations.

To obtain a standard curve, different amounts of standard thiocyanate solution were diluted to 5 ml with water, and 5 ml of freshly constituted ferric nitrate reagent added. The colour intensity was measured against a solution of 5 ml of reagent diluted with 5 ml of water. The Beer-Lambert law was found to hold over the concentration range investigated. The recovery of sodium thiocyanate from serum was tested by adding known amounts of the standard thiocyanate solution to pig sera. These were

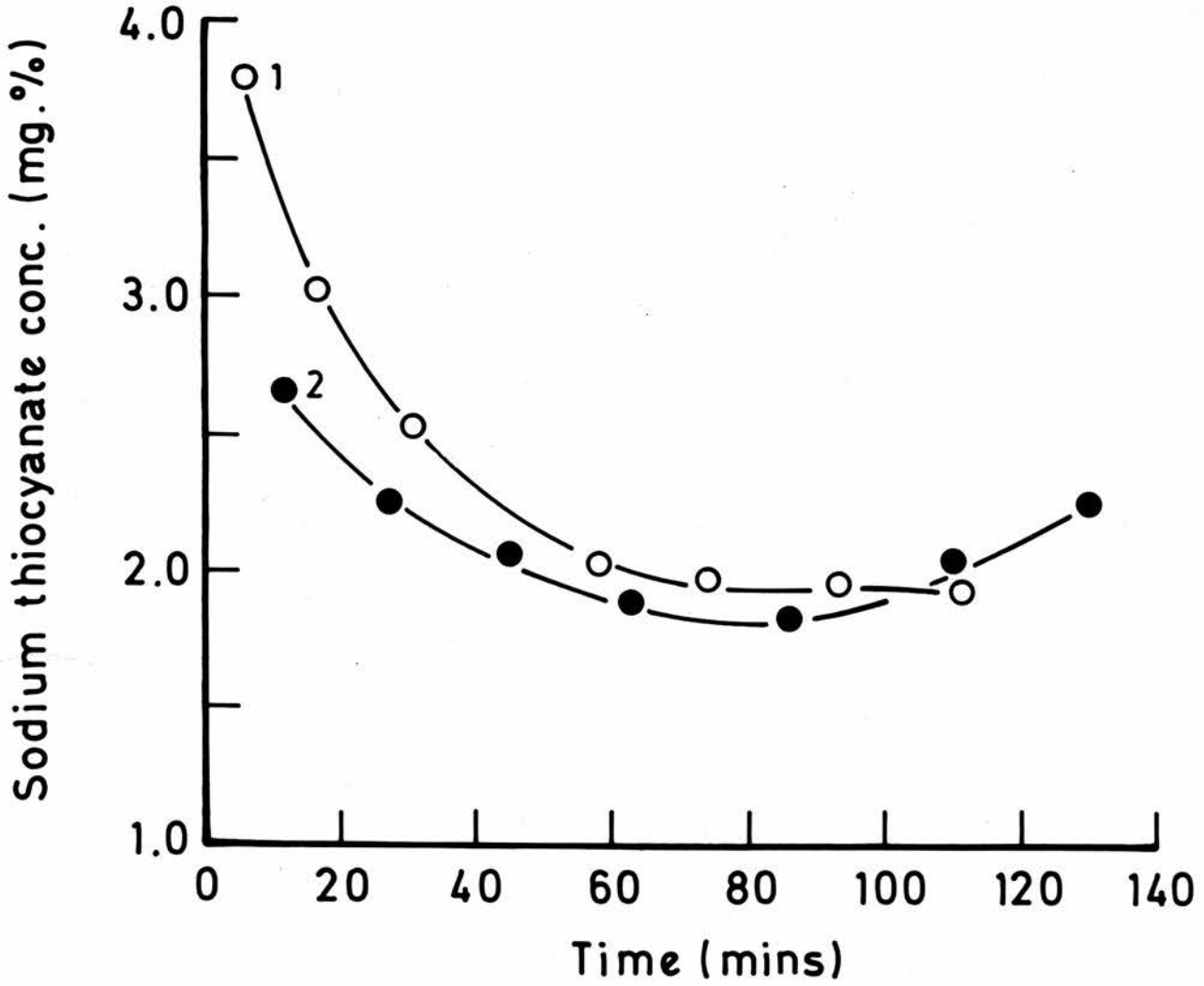


Fig. 8.2 Dilution Curves of Sodium Thiocyanate in one Sow.

treated in the normal way and their colour intensity compared with standard solutions. The mean recovery of thiocyanate from duplicate samples of six serum samples was 97.60% (S.D. = ± 1.873%) over the concentration range 0.5 to 6 mg%.

A further test was initiated to determine the extent of interference of D_2O, Evans Blue and urea on the estimation of thiocyanate. It was found that additions of these solutes to the thiocyanate solution did not interfere with the colorimetric estimation of thiocyanate.

(ii) Application of the method to sows. In the first pig, blood samples were taken up to 4 hours post-injection to determine the equilibration time. Fig. 8.2, curve 1, shows that the injected thiocyanate equilibrated in the body in about $1\frac{1}{2}$ to 2 hours. In a subsequent infusion of thiocyanate into this sow, dilution curve 2 was obtained (Fig. 8.2), which indicated that there was possibly some redistribution of thiocyanate in the circulation, after equilibrium had been attained. In the second sow, repeat estimates of the extent of thiocyanate distribution in the body were made on three successive days. The estimates of thiocyanate space were calculated from the equation given in Chapter 6, and they are shown in Table 8.2.

Table 8.2

Comparison between thiocyanate space and deuterium oxide space in two sows

	Thiocyanate space (litres)	Deuterium oxide space (litres)
Sow 1	100.6	109.6
Sow 2	68.2	75.38
	82.9	73.49
	66.5	73.06
Means and S.D's for Sow 2	72.5±7.36	73.98±1.008

From this table it can be seen that in sow 2 there was a great variation in the thiocyanate space from day to day. The values of the thiocyanate space were similar to the estimates of D_2O space which suggests that sodium thiocyanate considerably overestimated the extracellular water space, possibly by penetrating into the intracellular fluid space.

(iii) Discussion. From these results it was concluded that the thiocyanate dilution method for the estimation of extracellular water space in adult pigs was not reliable.

Although the technique was simple to operate, certain precautions were necessary. Addition of the reagent (ferric nitrate) to form a colour complex with the thiocyanate had to be made in artificial light, otherwise appreciable fading occurred. Also, the colour intensity had to be measured immediately after addition of the reagent. In the experience gained from this study, difficulty was often found in obtaining an uncontaminated sample of the serum.

In the first sow, thiocyanate was found to equilibrate in about $1\frac{1}{2}$ to 2 hours. In some preliminary test infusions, the dilution curves which were obtained, indicated that after equilibrium, redistribution and metabolism of the thiocyanate may have occurred.

The estimates of extracellular volume by thiocyanate were found to be extremely variable, and were often in excess of the anticipated value. Previous workers have also found that sodium thiocyanate overestimated extracellular volume (Flynn et al., 1968) in pigs and in human subjects (Doxiadis and Gairdner, 1948). In in vitro studies, Scheinberg and Kowalski (1950) found that a substantial proportion of thiocyanate became bound to a non-diffusible component of the blood. These workers concluded that equilibrium does not exist between serum thiocyanate and extravascular thiocyanate, because of this non-diffusible component.

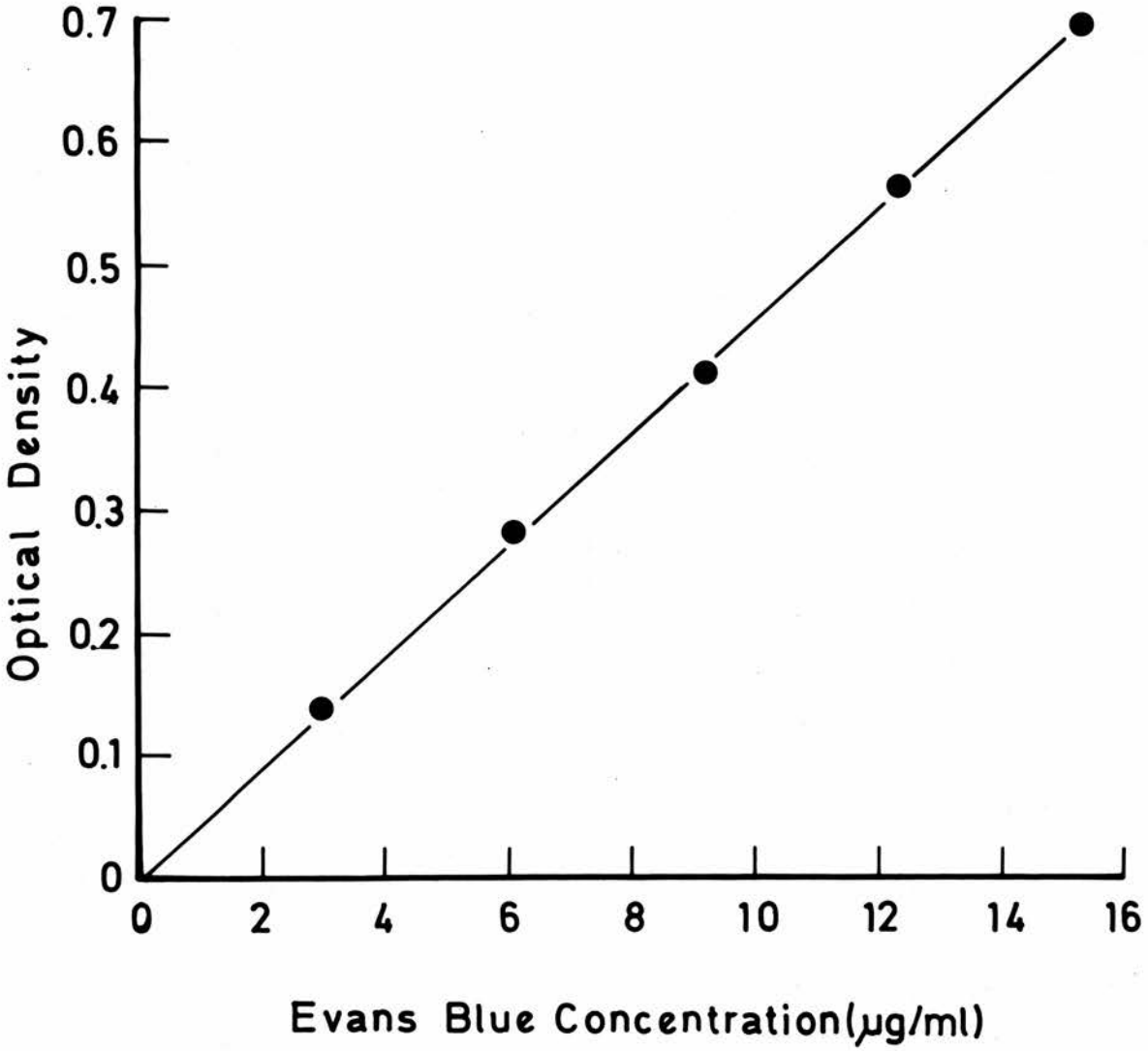


Fig. 8.3. Relationship Between Evans Blue Concentration And Optical Density

Rosenbaum and Lavietes (1939) found that thiocyanate became bound to serum lipids. These findings possibly explain the shape of the dilution curves obtained in this study and a differential recovery of the thiocyanate from the serum, during the mixing phase, could be responsible for the aberrant results.

(iv) Appraisal of the method and conclusions. The thiocyanate dilution technique was found to have a relatively simple analytical procedure. However, when the method was applied to sows, unreliable results were obtained. Repeat determinations on the same sow were extremely variable and often the estimates of extracellular space approached those of total body water determined by D_2O.

It was decided, on the basis of these results, not to include the method in further studies.

(c) The Evans Blue dilution technique

(i) Laboratory methodology. Preliminary investigations were conducted to determine the relationship between optical density and Evans Blue with increasing concentrations of the dye in plasma, and the recovery of the dye from plasma in the presence of urea and D_2O and thiocyanate.

Solutions of Evans Blue were constituted to cover a range of concentration from 0.003 mg/ml to 0.015 mg/ml in porcine plasma and distilled water. The solutions were measured at 620 nm. Fig. 8.3 shows that the optical density of Evans Blue in the plasma samples was related to their concentration, obeying the Beer-Lambert law. The recovery of Evans Blue from plasma, based on eight samples was 98.40% (S.D. = $\pm$ 1.704%). The addition of urea, D_2O and sodium thiocyanate to solutions of Evans Blue in water and plasma did not affect the optical densities of these solutions.

(ii) Application of the method to a sow. Estimates of plasma volume in one sow were made by back-extrapolation to determine the initial

volume of dilution of the Evans Blue before it left the circulation. Haematocrit readings taken at 0, 30 and 60 minutes after injection of the dye enabled estimates of blood volume to be made. The equation for this calculation is:-

$$BV = (100 \times PV)/(100 - H)$$

where BV = blood volume

PV = plasma volume

and H = haematocrit reading (mean of 30 and 60 minute readings)

The red cell volume was calculated as the difference between blood volume and plasma volume. The results are shown in Table 8.3.

Table 8.3

Estimates of blood volume, red cell volume and plasma volume by Evans Blue dilution in one sow

Replicate	Blood Volume (litres)	Red Cell Volume (litres)	Plasma Volume (litres)
1	10.78	3.66	7.12
2	10.93	3.83	7.10
3	10.70	3.75	6.95
4	11.20	3.90	7.30
5	10.03	3.33	6.70
Mean	10.53 ± 0.436	3.69 ± 0.199	7.03 ± 0.201

The estimate of plasma volume from Evans Blue was highly repeatable. The mean plasma volume estimate was 7.03 litres with a standard deviation of ± 0.201 litres, which is 2.84% of the mean. The maximum range was 0.60 litres which was between replicate 4 and 5.

(iii) Discussion. The estimates of plasma volume by Evans Blue were based on only the last five of the six blood samples taken. For each

determination it was found that the value of the first sample taken at about 10 minutes after injection did not fit the regression line as closely as did values of the last five samples. This suggests that the concentration of the Evans Blue in the plasma did not decline uniformly with time. This finding is in agreement with that of Anderson, McDonald and Elsley (1969) who found that the decline of Evans Blue in the plasma could be expressed by a quadratic equation. In addition, fluctuations in the jugular haematocrit during each determination were observed. Statistical analyses of the haematocrit readings showed that the readings at T_o were higher, ($P < 0.01$) than were later values. The 30- and 60-minute readings were also variable but no significant difference between them for the five replicates could be found. These findings are in agreement with those of Anderson, McDonald and Elsley (1969).

The estimates of blood volume obtained in this experiment were slightly higher than those obtained by Anderson, McDonald and Elsley (1969) in sows of similar weight. It is difficult to ascribe any reason for this, because it is not known if the Evans Blue method actually measures blood volume. If it is assumed that (a) the method does measure the true blood volume (b) blood volume is closely related to the size of the 'active' mass, then it follows that the sows used in the present study were leaner than those used in the study of Anderson, McDonald and Elsley (1969).

(iv) Appraisal of the method and conclusions. The Evans Blue dilution technique was simple and inexpensive to implement. Application of the method to one sow showed that it was highly repeatable, and the estimates of blood volume were slightly higher than those obtained in a previous study in which sows of similar weight were used. The results obtained here indicate that repeatable estimates of blood volume can be

made if the Evans Blue concentration of the first blood sample is ignored, and only the 30- and 60-minute haematocrit readings are used. Although there was no direct verification of the accuracy of the method, the results obtained here favour its inclusion in a later experiment.

Summary

(a) Urea space determinations were made in two sows. The initial concentration of urea in the body was calculated by back-extrapolation. The variation in the slope of the dilution curve indicated that there was either a variable endogenous production of urea or that the exogenous urea was metabolised at different rates after its injection. Comparison of the urea space with the D_2O space determined in the same pigs, revealed that there were large variations in the urea space during a period of three days. The method was therefore not considered sufficiently precise to be included in further investigations.

(b) Extracellular fluid space was estimated in two sows using sodium thiocyanate. Thiocyanate was found to equilibrate in the body in about $1\frac{1}{2}$ to 2 hours although the dilution curves obtained indicated that redistribution of the tracer may have occurred. In one sow in which repeat determinations of extracellular space were made, the values obtained were extremely variable. In both sows the estimated thiocyanate space was similar to the body water space as determined by D_2O dilution. The method was therefore not considered suitable for further investigation.

(c) The Evans Blue dilution technique was applied to only one sow. Repeat estimates were made over a period of three days. The estimates of plasma volume were calculated by back-extrapolation and the haematocrit value was calculated as the mean of the 30- and 60-minute readings. The estimates of blood volume, calculated from the plasma volume and the haematocrit values, were highly repeatable and were slightly higher than those obtained in a previous study, using sows of similar weight. The method is to be included in a further investigation.

CHAPTER 9

THE SIMULTANEOUS APPLICATION OF SELECTED INDIRECT TECHNIQUES TO THE LIVE PIG - EXPERIMENT 3

Introduction

A description of the second phase of the project in which several selected indirect techniques were simultaneously applied to 24 live pigs is given in this chapter. The methods were applied to each pig when it weighed about 90 kg.

Some of the techniques which had been investigated in the first phase of the project were included in this experiment. These were the potassium 42, deuterium oxide and Evans Blue dilution techniques.

In addition, several non-dilution techniques such as feed conversion, measurements of external dimensions, ultrasonics and visual appraisal were also included. Several post-mortem measurements were also taken on the chilled carcasses. These were carcass backfat depths and specific gravity.

Design of the experiment

Details of the animals used, their management and housing during the growing period were described in Chapter 6, together with the details of the techniques which were applied.

In this experiment, 12 castrated male pigs and 12 gilts were used. Twenty-four other animals (12 castrated males and 12 females) were used as replacement stock, so that if any of the experimental animals were found to be performing unsatisfactorily, they could be replaced by animals receiving the same dietary treatments.

Each of the 24 animals was selected from different litters at

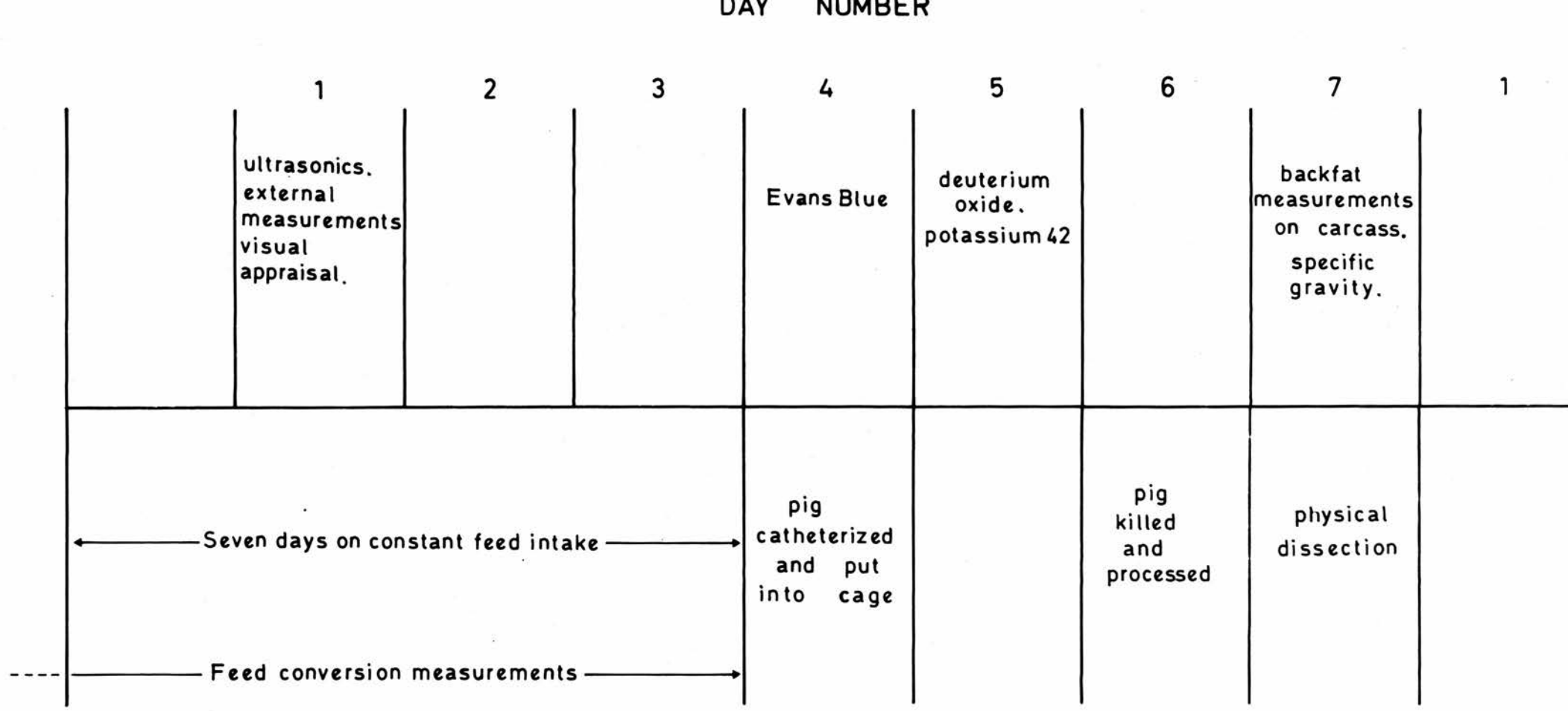


Fig. 9.1 Order of Application of Indirect Measurements to the Pigs in Experiment 3

about 20 kg liveweight. They were fed diet NRS1 (Appendix Table 1) until they reached approximately 25 kg liveweight. Each pig was then weighed on three successive days to obtain an accurate estimate of its initial liveweight. The pigs were reared from 25 kg to 90 kg along different growth curves which were intended to promote large differences in body fatness. Twelve dietary treatments were used. These were combinations of increasing protein concentration and increasing feed intake. Two females and two castrates were allocated to each of these treatments. The experimental design is shown in Table 9.1 and the composition of the two diets, X and Y, which were used is shown in Appendix Table 8.

Table 9.1

The design of experiment 3 and the allocation of the pigs to the dietary treatments

% Protein in the diet (Air-dry basis)	Feed intake ($g/kg^{0.73}/24$ h)											
	70		84		98		112		126		140	
	M	F	M	F	M	F	M	F	M	F	M	F
13 (Diet X)	13	12	9	8	24	7	17	16	6	5	4	3
22 (Diet Y)	15	14	11	10	23	22	21	20	19	18	2	1

M = castrated male

F = female

The indirect techniques were applied to each animal when it weighed about 90 kg. The programme which describes the order in which the measurements were taken on each animal is shown in Fig. 9.1.

The amount of work involved in the application of the indirect techniques and the analytical procedures did not allow more than one pig to be slaughtered each week.

Presentation of the results

The results of this experiment are presented in two parts.

In the first part the direct estimates of body composition of the experimental animals are presented and the relationships between the body constituents are discussed.

In the second part the indirect estimates of body composition are compared with the direct estimates. The indirect methods which were used are classed into three groups. In the first group are the dilution techniques, ^{42}K, D_2O and Evans Blue. In the second group are the non-dilution techniques, external measurements, ultrasonic measurements and visual appraisal. The feed conversion ratio and specific gravity techniques are in the third group.

In formulating quantitative relationships between the indirect and direct estimates of the various body components, it was found on preliminary inspection of the data that there was no improvement in accuracy by including quadratic terms into the prediction equations. Also there were no sex differences in the relationships between the indirect and direct estimates for each predictor and therefore the regression equations were based on 24 data points. The first series of regression equations took the form:-

$$\hat{y} = ax_1 + bx_2 + c$$

where y = the direct measurement of the body component

$\hat{y}$ = the regression estimate of y

x_1 = one of the measurements used to estimate y

x_2 = the liveweight of the animal at slaughter

c = constant term

and a, b, and c are constants estimated by regression analyses on the values of y, x_1 and x_2. Liveweight was only included in the equations when it substantially improved the relationships between the indirect and direct estimates.

Results - I

(a) The body composition of the pigs

The relevant carcass analysis data on the 24 animals are given in Appendix Tables 9a and 9b.

The mean liveweight at slaughter for the 24 animals was 83.87 kg (S.D. = ± 5.113 kg). It was hoped that the weight at slaughter would be constant to avoid the effects of liveweight differences confounding with the prediction equations, but the maximum range of values recorded was 19.99 kg. It is assumed that variation in the starting weights, deviations from the anticipated growth rates and a strict adherence to the slaughtering programme resulted in this spread of the slaughter weights. In the prediction equations, the effects of these liveweight differences have been taken into consideration.

The mean empty body weight of the 24 animals was 80.62 kg (S.D. = ± 4.574 kg). Empty body weight was closely correlated with liveweight ($r = 0.976$), the variation being due mainly to the amount of gut-fill. The mean gut-fill at slaughter was 2.67 kg (S.D. = ± 0.742 kg). From these data it is difficult to tell whether the attempt at standardising gut-fill by feeding a standard intake for one week had any effect.

The total body lipid was calculated as the sum of the weights of lipid in each of the three carcass divisions of the body, the physically dissected side, the jointed side, and the middle of the animal. There was a large range in the lipid content of the empty body. It ranged from 17.05% to 34.45% and the fattest pigs were those which were reared on the high feed intake/low protein diet. There were no significant differences ($P > 0.05$) between the lipid contents of the castrated males and females, although there was a trend for the castrates to be slightly more obese than the females. The amount of dissectible fat (subcutaneous fat) was highly correlated with the body lipid ($r = 0.958$). The concentrations of water, protein, potassium and ash in the fat-free body mass were remarkably constant (Table 9.2).

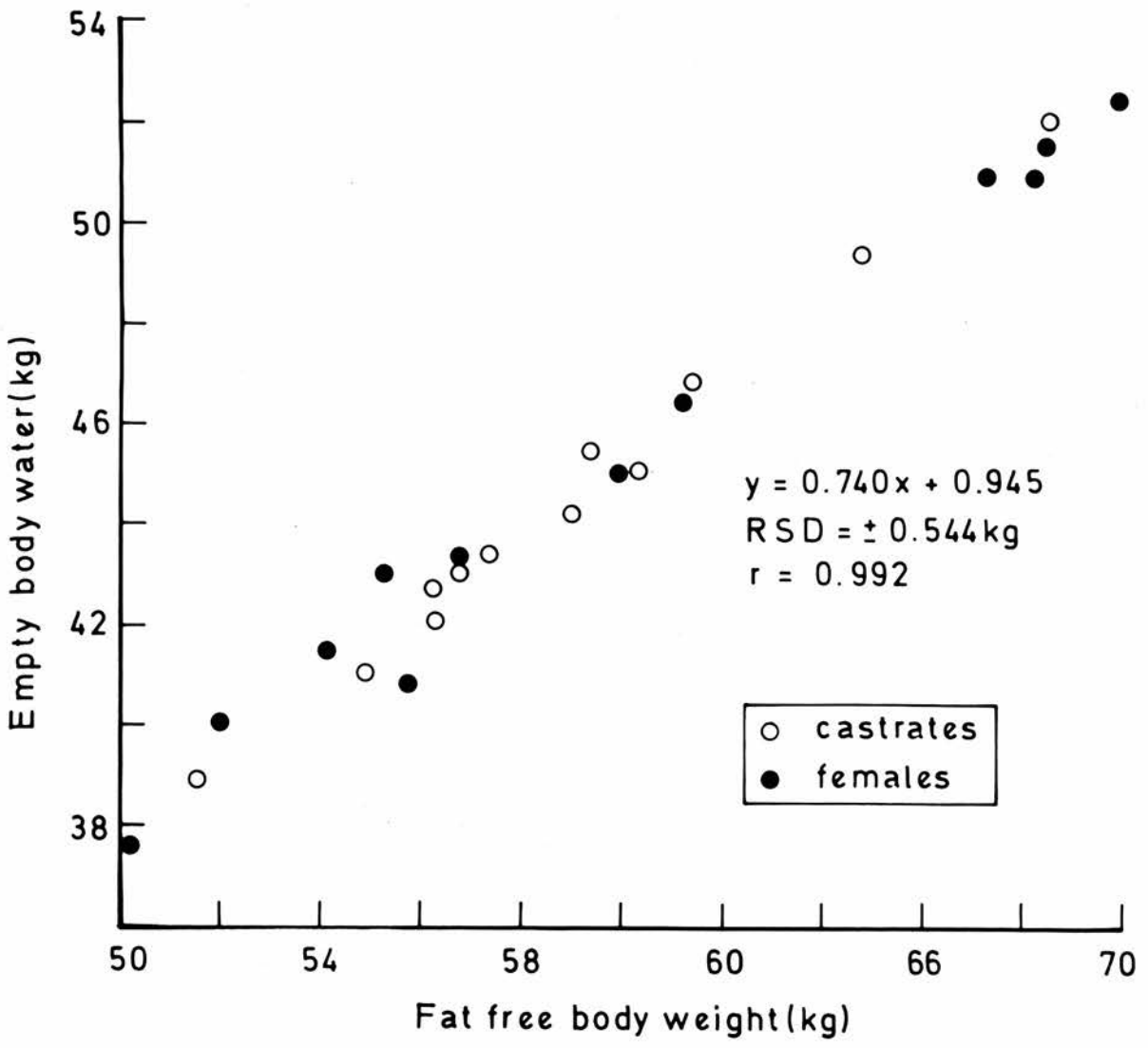


Fig.9.2 The Relationship Between Empty Body Water(kg) and the Fat Free Body Weight(kg).

Table 9.2

The composition of the fat-free body mass (FFBM) and the fat-free dry matter (FFDM)

FFBM

	Water		Protein		Potassium		Ash	
	%	r	%	r	%	r	%	r
Mean	75.61	0.992	20.14	0.948	0.269	0.975	4.23	0.897
	±		±		±		±	
Standard Deviation	0.955		0.727		0.0062		0.248	

FFDM

	Protein		Potassium		Ash	
	%	r	%	r	%	r
Mean	82.59	0.993	1.103	0.984	17.34	0.954
	±		±		±	
Standard deviation	1.189		0.0253		0.615	

The empty body water constituted 75.61 ± 0.955% of the fat-free mass (see Fig. 9.2). The potassium content of the fat-free mass was also remarkably constant (0.269 ± 0.0062%) although it was more closely associated with the fat-free dry matter (r = 0.984).

Despite the large range in the age (153 to 290 days), body fatness (17 to 35%), and fat-free weight (50.17 kg to 69.86kg) of these animals, these factors did not account for any of the variability in the proportions of water and potassium in the fat-free body. The protein and ash contents of the fat-free mass were rather more variable, and both these constituents were more closely associated with the fat-free dry material (Table 9.2). This suggests that within the electrolyte/protein/water complex of the body, there are variable amounts of water associated with the protein and electrolyte fractions. The interrelationships of these constituents of

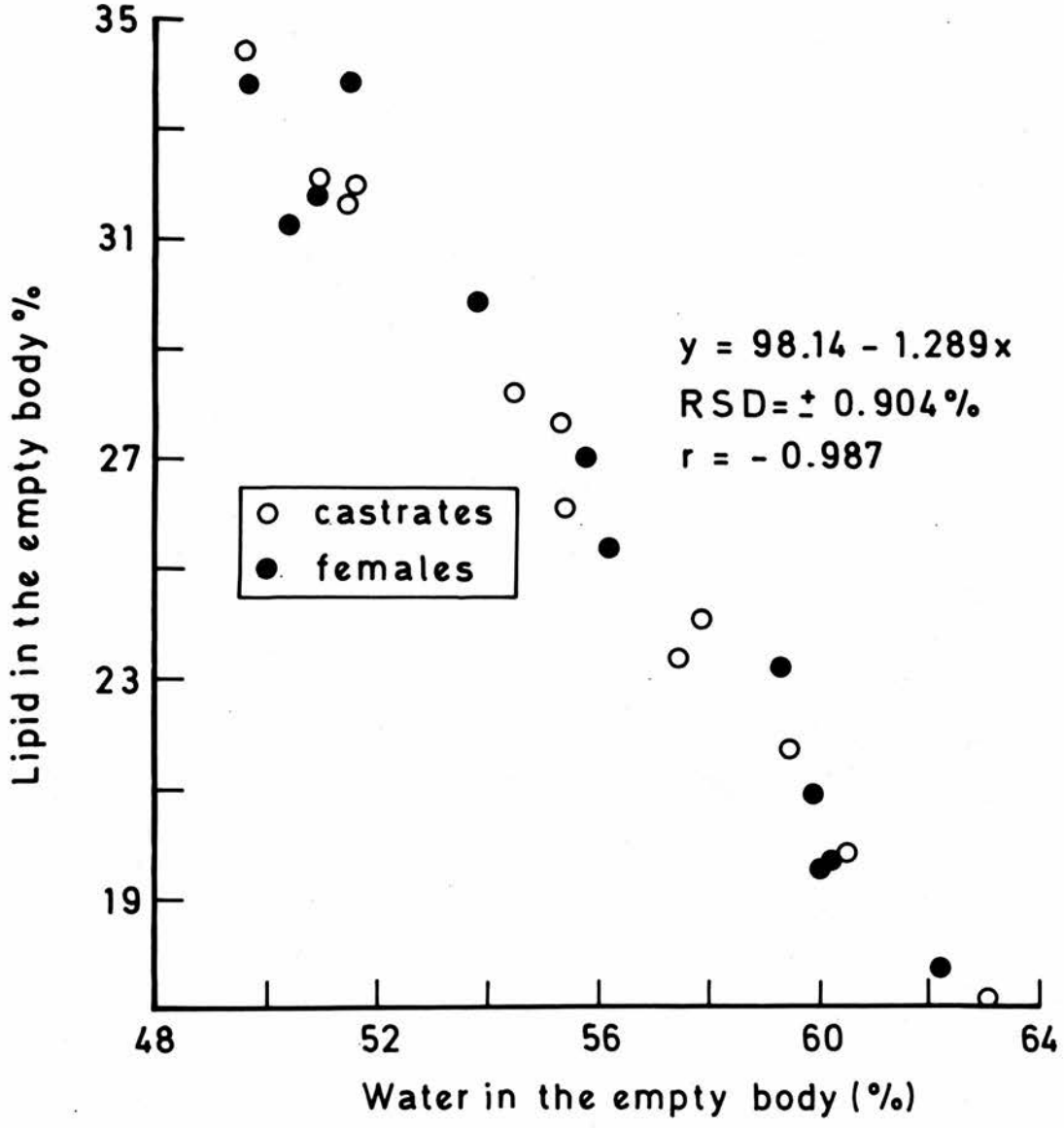


Fig. 9.4 The Relationship Between the Proportions of Lipid and Water in the Empty Body.

the pigs used in this study are shown in Fig. 9.3.

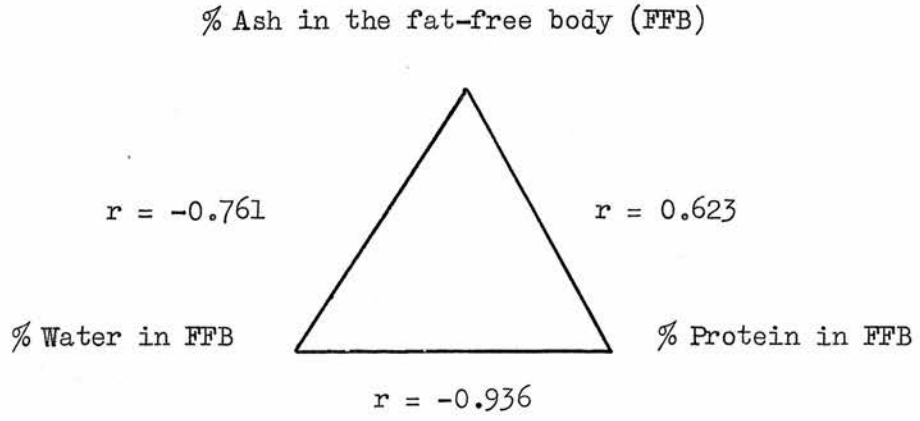


Fig. 9.3. Interrelationships between the proportions of ash, water and protein in the fat-free body

The proportion of water in the empty body was highly correlated with the proportion of lipid in the empty body (r = 0.987) (see Fig. 9.4). This finding is in agreement with previous studies (Clawson, Sheffy and Reid 1955; Reid et al., 1968) which have also demonstrated a close inverse relationship between these constituents of the body.

The weights of the dissectible components of the body were calculated from the weights of the dissectible material obtained from the physically dissected side (PDS) of the carcass. The maximum difference in weights between the two carcass sides was 1.80 kg. Dissection losses were small, averaging 1.63% (S.D. = 1.022%) of the side and these were assumed to be moisture.

The dissectible lean (DL) had a mean weight of 34.65 kg (S.D. = 3.545 kg) and formed on average 41.30% of the liveweight and 58.38% of the fat-free weight.

The dissectible fat (DF) which was composed entirely of subcutaneous fat had a mean weight of 17.71 kg (S.D. = 3.371 kg). It formed on average 83.4% of the total lipid weight of the body. The correlation between these two variables was found to be 0.958.

The dissectible fat-free mass (DFFM) defined as the empty body weight less the weight of the subcutaneous fat had a mean weight of 61.84 kg (S.D. = 4.835) and formed 73.75% of the liveweight. It was found to be on average 2.45 kg larger than the chemically-defined fat-free mass, because it also included the intramuscular and intermuscular fat.

The commercial carcass yield was defined as the empty body weight, less the weight of the head, internal organs, alimentary tract and chine. Its mean weight was 55.95 $\pm$ 4.167 kg and formed on average 66.68% of the liveweight.

(b) <u>The relationships between the body components and liveweight</u>

The regression equations between the chemical components of the body and liveweight for the 24 animals are shown in Table 9.3. All the major chemical components of the body, except total body lipid were significantly correlated with liveweight. The non-significant relationship between the total body lipid and liveweight demonstrates how nutritional treatments can determine the relative proportions of fat in the body. Lipid is considered to be a buffer between the energy supply and the immediate energy requirements of the animal (Fowler, 1967).

The relationships between liveweight and empty body water, crude protein, fat-free mass, fat-free dry matter were very similar (see Table 9.3) and it was calculated that the unexplained variability in these body components, after accounting for liveweight, was 61.9%, 61.3%, 60.4%, and 62.5%, respectively.

Table 9.3

Regressions of the chemical components of the body on liveweight

Independent variable (x) = Liveweight at slaughter (kg) (n = 24)

Dependent variable (y) kg	Regression Equation	R.S.D. (kg)	r
Empty body water	y = 0.520x + 1.287	3.465	0.617
Total body lipid	y = 0.163x + 7.561	4.693	0.179 (N.S.)
Total crude protein	y = 0.156x - 1.118	1.027	0.622
Fat-free body weight	y = 0.710x - 0.200	4.590	0.629
Fat-free dry matter	y = 0.190x - 1.483	1.288	0.612

Table 9.4

Regressions of the dissectible components of the body on liveweight

Independent variable (x) = Liveweight at slaughter (kg) (n = 24)

Dependent variable (y) kg	Regression Equation	R.S.D. (kg)	r
Dissectible fat	y = 0.198x + 1.107	3.289	0.300 (N.S.)
Dissectible lean	y = 0.463x - 4.217	2.696	0.669
Dissectible bone	y = 0.029x + 2.198	0.503	0.290 (N.S.)
Commercial carcass yield	y = 0.715x - 4.049	2.042	0.878
Dissectible fat-free mass	y = 0.682x + 4.620	3.422	0.722

The relationships between the dissectible components of the body and the liveweight at slaughter are given in Table 9.4.

The amounts of dissectible lean, fat-free material and the commercial carcass were closely correlated with the total weight of the animal, presumably because they each formed a major proportion of the body weight.

Dissectible fat was not significantly correlated with live weight. The non-significant relationship between the dissectible bone and live weight was not a surprising result. In this experiment the dissectible bone included only the bone of both sides of the carcass (PDS and JS), and did not include the hock bones, chine or the skull bones.

Results - II

(a) The prediction of body composition by dilution techniques

Potassium 42

Estimates of exchangeable potassium (K_e) were available for 22 pigs. The specific activity values of plasma and urine at 22 to 26 hours post-injection were used to determine K_e. For the remaining two pigs, the activity of the potassium 42 before its injection was considered too low for accurate analyses to be made.

Corrections were made for the loss of the label into the gut contents, faeces and the urine. The loss of label into each of these three excretion products in the equilibrium period amounted to 1.14% ± 0.497%; 0.19% ± 0.183% and 2.41% ± 1.352%, respectively, of the injected label. The individual values are given in Appendix Table 10. The mean value for the loss of label into the faeces, included the results from five pigs which voided no faeces during the equilibration period.

The computed values of the exchangeable potassium from the plasma (K_{ep}) and the urine (K_{eu}) for each pig, were found to be similar and also

similar to the chemical estimate of body potassium (K_c). The mean values and standard deviations for these three parameters are given in Table 9.5 and the individual values for each of the 22 animals are presented in Appendix Table 11.

Table 9.5

Comparisons between the estimates of body potassium (K_c) and the exchangeable potassium (g) predicted from the urine specific activity (K_{eu}) and the plasma specific activity (K_{ep}) (n = 22)

K_c		K_{eu}		K_{ep}	
Mean	S.D.	Mean	S.D.	Mean	S.D.
159.5	± 16.44	155.7	± 14.25	154.2	± 14.50

These results can be compared to those obtained in experiment 1, in which a large variation between the values of K_{ep} and K_{eu} was found.

The regression equations relating the estimates of exchangeable potassium to the chemical potassium of the body for the 22 pigs are given in Table 9.6.

Table 9.6

Regressions of chemical potassium (K_c) on exchangeable potassium (K_{ep} or K_{eu}) (n = 22)

Dependent variable (g)	Independent variable	Regression equation	RSD (g)	r
K_c	K_{ep}	$y = 1.081\ K_{ep} - 7.20$	5.11	0.953
K_c	K_{eu}	$y = 1.086\ K_{eu} - 9.71$	5.66	0.942

From the mean values of K_{ep}, and K_c, it was calculated that 96.7% of the body potassium was measured by K_{ep} in 22-26 hours. For K_{eu} the proportion was 97.6%.

The prediction of the chemical components of the body by exchangeable potassium. The similarity of the regression equations between each of the estimates of exchangeable potassium and the chemically-determined potassium (Table 9.6) showed that there was little difference in the predictive accuracy of either K_{ep} or K_{eu}. In order to avoid repetition in the text, only the plasma estimate of exchangeable potassium, K_{ep}, will be used for predicting the weights of body components.

In almost all of the regressions between the body components and K_{ep}, the inclusion of liveweight as an independent variable did not improve the accuracy of prediction.

The regression equations between the chemical components of the body and K_{ep} are shown in Table 9.7. The relationship between K_{ep} and the fat-free body mass was remarkably close ($r = 0.976$). From the regression equation it can be seen that by using ^{42}K dilution the weight of the fat-free material in the live animal can be estimated to $\pm$ 1.347 kg. This represents 2.3% of the mean fat-free weight.

Table 9.7

The prediction of the chemical components of the body by exchangeable potassium (K_{ep})

Independent variable $x_1 = K_{ep}$ (g) x_2 = Liveweight (kg) (n = 22)

Dependent Variable (y) (kg)	Regression Equation	R.S.D. (kg)	R
Fat-free mass	$y = 0.405\ x_1 - 3.210$	1.347	0.976
Empty body water	$y = 0.301\ x_1 - 1.629$	1.031	0.974
Fat-free dry matter	$y = 0.104\ x_1 - 1.571$	0.722	0.906
Crude protein	$y = 0.085\ x_1 - 1.177$	0.540	0.920
Total body lipid	$y = 16.483 - 0.383\ x_1 + 0.762\ x_2$	1.599	0.950

The relationship between the empty body water and K_{ep} was similar to that described above ($r = 0.974$). This was not surprising, because the empty body water also formed a constant proportion of the fat-free body mass.

Similar relationships were obtained between the fat-free dry matter and K_{ep} ($r = 0.906$) and crude protein and K_{ep} ($r = 0.920$). The addition of liveweight as an independent variable, significantly improved the relationship between exchangeable potassium and body lipid. Differences in liveweight presumably reduced the normally close inverse relationship between the percentage of body lipid and the percentage of potassium in the body. From the regression equation the total lipid in the live animal could be predicted from K_{ep} with a standard deviation of 1.599 kg which is 7.5% of the mean.

The prediction of the dissectible components of the body, by exchangeable potassium. The regressions of the weights of dissectible components on K_{ep} are presented in Table 9.8.

Table 9.8

The prediction of the dissectible components of the body by exchangeable potassium (K_{ep})

Independent Variable $x_1 = K_{ep}$ (g) x_2 = Liveweight (kg) (n = 22)

Dependent Variable (y) (kg)	Regression Equation	R.S.D. (kg)	R
Dissectible fat-free mass	$y = 0.323\ x_1 + 11.829$	1.836	0.934
Dissectible lean	$y = 0.208\ x_1 + 2.371$	2.144	0.822
Dissectible fat	$y = 6.701 - 0.250\ x_1 + 0.592 x_2$	1.642	0.889
Commercial carcass yield	$y = 0.131\ x_1 + 35.679$	3.962	0.441

The correlation coefficients were not as high as those obtained between the chemical components and K_{ep}, and possibly reflected the heterogenous composition of the dissectible components. A comparison of the R.S.D. of the regression of DFFM on K_{ep} (R.S.D. = 1.836 kg) with that of FFM on K_{ep} (1.347 kg) suggests that the amount of fat in the DFFM contributed substantially to the larger error of estimate by K_{ep}.

Similarly, the relationship between dissectible lean and K_{ep} was not very close, the correlation coefficient was 0.822 and the R.S.D. of the regression was 2.144 kg. One possible reason for this poor relationship is that the dissectible lean did not represent the entire mass of 'lean-tissue' because 'lean deposits" such as the internal organs, alimentary tract and the musculature of the head were not included.

The relationship between the dissectible fat and K_{ep} was relatively poor, even when liveweight was included. The multiple correlation coefficient was 0.889 and the R.S.D. was 1.642 kg which was 9.21% of the mean value of the dissectible fat. The relationship between the yield of the commercial carcass and K_{ep} was also relatively poor. The correlation coefficient was 0.441 and the R.S.D. of 3.962 kg was 7.09% of the mean value. The commercial carcass consisted of large quantities of fat in addition to lean tissue, and its heterogenous composition may have contributed to the large error of estimate. A comparison of this regression equation with that of the commercial carcass on liveweight showed that estimates of exchangeable potassium were of little value in predicting the yield of saleable carcass.

Deuterium oxide

In each of the 24 pigs the deuterium oxide (D_2O) space was calculated from the following equation:-

$$D_2O \text{ space} = \frac{\text{Amount of } D_2O \text{ injected x Purity}}{\text{Density of } D_2O \text{ at } 20^{\circ}C \text{ x Equilibrium concentration}}$$

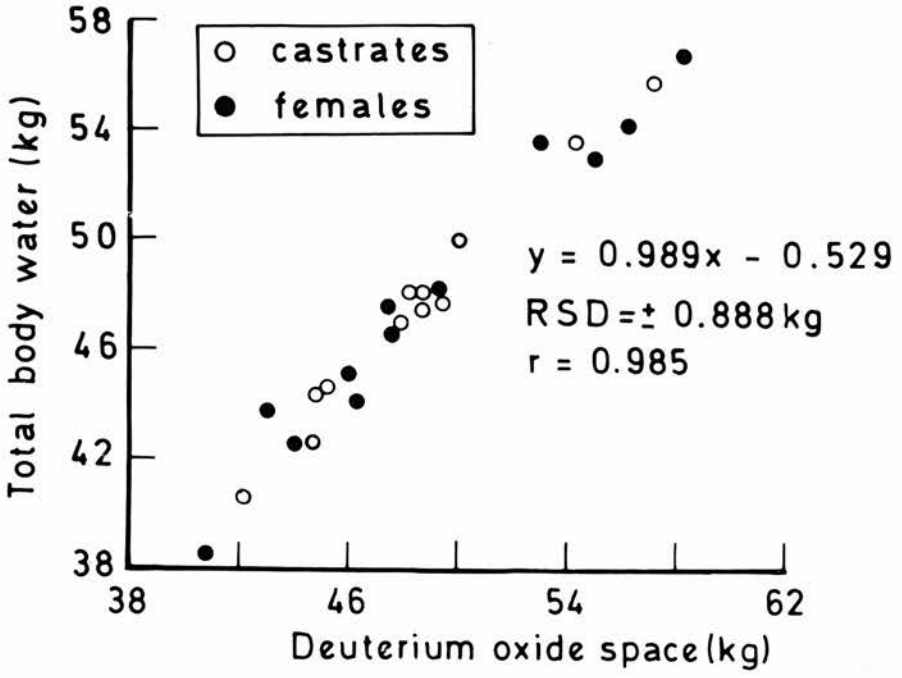


Fig. 9.5 Relationship Between Deuterium Oxide Space and Total Body Water.

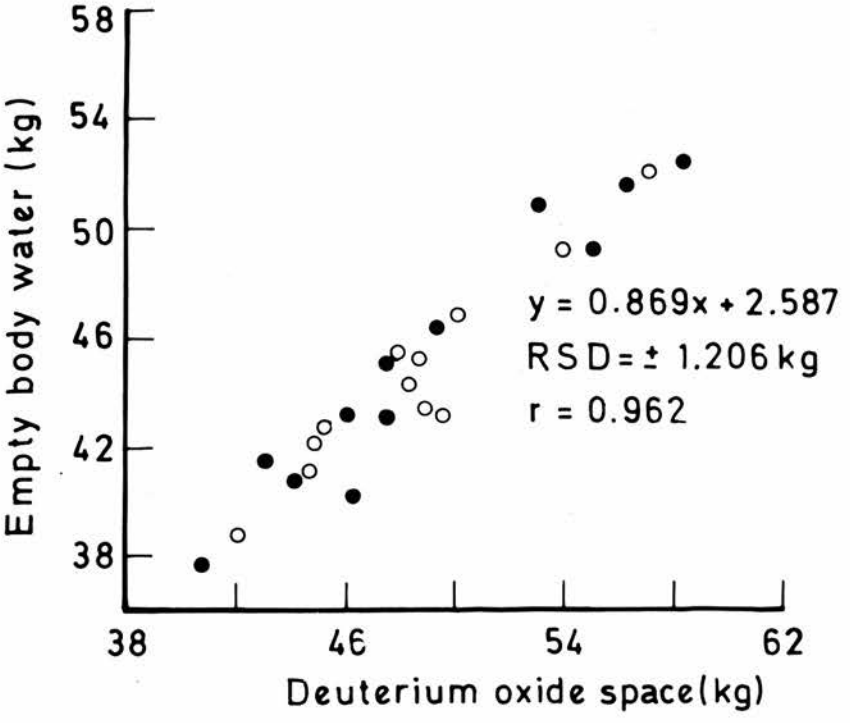


Fig.9.6 Relationship Between Deuterium Oxide Space and Empty Body Water

The equilibrium concentration of D_2O was calculated from the mean of three plasma values obtained between 2 to 3 hours after injection of the isotope. No corrections were made for the loss of the isotope into the gut contents, faeces and urine.

The estimates of D_2O space were compared with empty body water space and the total body water space obtained by dessication (Table 9.9).

Table 9.9

Comparisons between the mean estimates of the deuterium oxide space (kg) with those of empty body water (kg) and total body water (kg) (n = 24)

D_2O space		Empty body water		Total body water	
Mean	S.D.	Mean	S.D.	Mean	S.D.
48.67	± 4.766	44.89	± 4.307	47.65	± 4.797

The individual results for each of the 24 animals are presented in Appendix Table 12.

There are two main aspects of the data presented in Table 9.9. Firstly, the D_2O space appeared to be more closely associated with the total body water than with the empty body water. The respective regression equations are given in the following table (Table 9.10). The relationships are also shown in Figs. 9.5 and 9.6.

Table 9.10

Relationships between the deuterium oxide space (kg), empty body water (kg) and total body water (kg) (n = 24)

EBW = 0.869 D_2O space + 2.587 R.S.D. = ± 1.206 kg r = 0.962

TBW = 0.989 D_2O space - 0.529 R.S.D. = ± 0.888 kg r = 0.985

The second aspect of the results was that in 21 pigs, D_2O over-estimated the TBW space by an average of 1.30 kg which was 1.56% of the

mean liveweight of the pigs at slaughter. These observations endorse the results for D_2O dilution obtained in experiment 1, from which it was concluded that the D_2O had also equilibrated in the water of the intestinal tract and bladder, and had possibly exchanged with some of the more labile hydrogen ions of the body.

Although the estimate of the D_2O space was more closely related to the total body water, the interest in this relationship is only academic. Included in the total body water is the intestinal water which is not physiologically related to the size of any body component. It is also extremely variable depending on the size and nature of the gut contents and to some extent on the level of starvation imposed on the animal.

The prediction of the chemical components of the body by the D_2O space. The regression equations computed between the various body components and the D_2O space indicated that the method was useful for predicting the composition of live animals. However, the potential accuracy of the method was possibly not realised because, as was shown in Table 9.10, the D_2O space was more closely related to total body water than to the empty body water. The close relationships which were found to exist between EBW and other body components were not found with TBW.

In Table 9.11, the prediction of several chemical components of the body by D_2O are shown. The relationship between the fat-free body mass and the D_2O space was fairly close. The correlation coefficient was 0.948 and the R.S.D. of the regression was 1.876 kg which was 3.2% of the mean fat-free body weight.

The relationships between the D_2O space and the fat-free dry matter ($r = 0.837$) and the crude protein ($r = 0.859$) were not very close, which suggests that body water is not closely associated with these components of the body.

The inclusion of liveweight into the regression significantly improved the relationship between the total body lipid and the D_2O space. The R.S.D. was reduced from 3.836 kg to 1.420 kg which was 6.69% of the mean body lipid weight. Liveweight presumably accounted for the variability in the inverse relationship between the proportions of water and lipid in these pigs.

Table 9.11

The prediction of the chemical components of the body from deuterium oxide space (n = 24)

Independent variable X_1 = D_2O space (kg) x_2 = Liveweight (kg)

Dependent variable (kg) (y)	Regression equation	R.S.D. (kg)	R
Empty body water	$y = 0.869\ x_1 + 2.595$	1.206	0.962
Total body lipid	$y = 4.759 - 1.216\ x_1 + 0.904\ x_2$	1.420	0.957
Total crude protein	$y = 0.231\ x_1 + 0.715$	0.672	0.859
Fat-free mass	$y = 1.149\ x_1 + 3.470$	1.876	0.948
Fat-free dry matter	$y = 0.280\ x_1 + 0.882$	0.891	0.837

The prediction of the dissectible components of the body by the D_2O space. In general, the relationships between the dissectible body components and the D_2O space (Table 9.12) were more variable than those between the chemical body components and D_2O space. This was possibly because of the heterogenous composition of the dissectible components, and also because they represented only certain proportions of the chemically-defined components.

Table 9.12

The prediction of the dissectible components of the body from deuterium oxide space (n = 24)

Independent variable x_1 = D_2O space (kg) x_2 = Liveweight (kg)

Dependent variable (kg) (y)	Regression equation	R.S.D. (kg)	R
Dissectible fat	$y = 0.714\ x_2 - 0.846\ x_1 - 0.969$	1.066	0.953
Dissectible lean	$y = 0.596\ x_1 + 0.563$	2.166	0.802
Dissectible fat-free mass	$y = 0.955\ x_1 + 15.380$	1.672	0.941
Commercial carcass yield	$y = 0.387\ x_1 + 37.100$	3,820	0.443

Dissectible fat was only poorly correlated with D_2O space (r = 0.488) but the inclusion of liveweight into the regression significantly improved the relationship (R = 0.953). The R.S.D. of the multiple regression was 1.066 kg which was 6.02% of the mean dissectible fat weight.

The relationship between dissectible lean and D_2O space was not very close (r = 0.802) possibly because the lean fraction of the body represented only a variable proportion of the total water-soluble mass of the body. In contrast the relationship with the dissectible fat-free body mass was much closer (r = 0.941), and the R.S.D. of the regression was only 1.672 kg which was 2.70% of the mean value.

The correlation between D_2O space and the yield of commercial carcass was 0.443 indicating that about only 20% of variability in this component was explained by the D_2O space. Indeed, a comparison of the R.S.D. obtained here with that of the regression of the yield of commercial carcass on liveweight suggests that D_2O estimations were poor in predicting the weight of this component in the live animal. This was not surprising because this component included significant proportions of fat, with which D_2O did not equilibrate.

Evans Blue

The estimate of plasma volume (PV) for each of the 24 pigs was calculated by the back-extrapolation procedure outlined in Chapter 8. The mean values and standard deviations are presented in Table 9.13, together with those of red cell volume and blood volume. The individual values are given in Appendix Table 13.

Table 9.13

The mean values and standard deviations of certain blood parameters (n = 24)

Measurement	Units	Value	Standard Deviation
No. of animals	-	24	-
Blood volume	Litres	7.398	± 0.706
Plasma volume	Litres	4.886	± 0.414
Red cell volume	Litres	2.511	± 0.346
Haematocrit	%	34.1	± 0.1
Liveweight	kg	83.87	± 5.11

The blood volume (B.V.) of each pig was calculated from the equation B.V. = 100 x P.V./ (100 - H) where H was the mean haematocrit value of the blood samples taken at 30 and 60 minutes after injection of the dye. As in experiment 2 it was found that the Evans Blue concentration of the first plasma sample was invariably above the regression line, based on the values of the last five samples taken during the hour following injection. This suggests that the dye was lost from the circulation at a non-uniform rate. It was also found that the haematocrit reading of the blood sample taken at zero time was significantly higher ($P < 0.01$) than the reading of either the 30 minute and 60 minute samples. The mean haematocrit values of blood samples withdrawn at three time intervals after the injection of

the dye are shown in Table 9.14.

Table 9.14

Haematocrit values of blood samples taken at three different times after the injection of Evans Blue (n = 24)

Sampling time (mins after injection)	Mean (%)	SD (%)
0	34.9	± 3.08
30	34.2	± 2.26
60	34.1	± 2.06

There were no significant differences between the haematocrit values taken at 30 and 60 minutes after injection of the dye. There was also found to be less variation in the haematocrit readings of the 30- and 60-minute samples than in the sample taken at zero time. The red cell volume of each pig was computed as the difference between the total blood volume and plasma volume. In these calculations, no corrections were made for trapped plasma.

The values obtained in this experiment were compared with those of previous studies. The estimates of red cell volume, plasma volume and blood volume were expressed on a liveweight basis. Comparisons were made only with pigs of a similar weight range (Table 9.15).

Table 9.15

Comparisons of estimates of blood parameters obtained in this investigation with those of other studies

Author	Live-weight (kg)	Red cells/kg (ml)	Plasma/kg (ml)	Blood/kg (ml)	Jugular haematocrit (%)
Present study	83.9	29.9	58.3	88.2	34.1
Hansard, Sauberlich and Comar (1951)*	82.7	20.5	31.5	52.0	39.5
Bush et al.(1955)*	80.0	23.7	37.8	61.5	38.5
Doornenbal, Asdell and Wellington (1962)†	97.0	19.9	31.4	51.3	39.0

*Phosphorus 32 method. † Chromium 51 method.

The data presented in Table 9.15 clearly demonstrate that the estimates of blood volume made in the present study were considerably higher than those obtained in previous investigations. A similar trend was also noted for the estimates of red cell volume and plasma volume. The methodology of the present study differed from the other studies in that the measurements of plasma volume were made directly by dye dilution, whereas previously it was calculated from the estimates of red cell volume. It has already been pointed out by Anderson, McDonald and Elsley (1969) that considerable inaccuracies can result in the calculation of P.V. from R.C.V. and vice versa. From their data it appeared that the most likely source of error was the variation between the large vessel haematocrit and the whole body haematocrit. They implied that the degree of splenic contraction, initiated by excitement, was the source of this variation. In the studies of Hansard, Sauberlich and Comar (1951) and Doornenbal, Asdell and Wellington (1962), blood samples were collected by venipuncture from conscious animals. This would almost certainly have caused the spleen to contract, which could have resulted in the large vessel haematocrit being higher than the mean body haematocrit. The estimates of plasma volume reported by these authors may therefore have been below the true value.

In the present study, the animals were conscious when the blood was withdrawn, although no undue excitement was caused because cannulation was used. However, it is possible that the spleen could have been fully dilated at the time of sampling due to the residual effects of trichloroethylene which was administered to the pigs about 5 hours prior to this study. It is possible, therefore, that blood volume was overestimated due to the large vessel haematocrit being less than the mean body haematocrit. Reeve, Gregerson, Allan and Sear (1953) reported that dilation of the spleen due to nembutal anaesthesia produced a large vessel

haematocrit 10% less than the mean body haematocrit. In addition, in the earlier studies referred to, inaccurate estimates of the red cell volume may have been made because of incomplete mixing of the tagged red cells within the blood system. This also would result in inaccuracies in the estimation of blood volume. Kraintz, De Boer, Smith and Huggins (1958) showed that the estimation of red cell volume from the dilution of marked red cells 10 to 15 minutes after injection, resulted in an underestimate of 11.3 $\pm$ 3.8% due to incomplete mixing of the labelled red cells throughout the blood in the spleen.

One of the reasons for the disagreement between the results of the present experiment with those of previous studies may have been due to differences in the body composition of the animals. Due to the lack of a better reference body on which to base these inter-study comparisons, liveweight was used. For a given liveweight, there may be different proportions of fat-free material and lipid.

The data obtained on the chemical composition of the pigs in this study permitted a comparison of the relationships between blood volume and liveweight and blood volume and fat-free mass. Table 9.16 shows these regression equations.

Table 9.16

Comparison of the relationships between blood volume and liveweight, and blood volume and the fat-free body (n = 24)

Independent variable (x) (kg)	Regression equation	R.S.D. (litres)	r
Liveweight	y = 0.096 x - 0.684	0.517	0.698
Fat-free mass	y = 0.100 x + 1.448	0.414	9.819

The equations presented in this table indicate that the proportion of body fat in these pigs was a major source of the variability in the relationship between blood volume and liveweight.

The prediction of the chemical components of the body by blood volume estimates. The relationships between blood volume and the chemical components of the body are presented in Table 9.17.

Table 9.17

The prediction of the chemical components of the body by blood volume estimates (n = 24)

Independent variable (x_1) = Blood volume (litres), x_2 = Liveweight (kg)

Dependent variable (y) (kg)	Regression equation	R.S.D. (kg)	R
Empty body water	$y = 4.927\ x_1 + 8.453$	2.598	0.808
Total body lipid	$y = 0.711\ x_2 - 5.685\ x_1 + 3.673$	3.744	0.642
Total crude protein	$y = 1.490\ x_1 + 0.939$	0.750	0.820
Fat-free mass	$y = 6.702\ x_1 + 9.804$	3.385	0.819
Fat-free dry matter	$y = 1.774\ x_1 + 1.363$	1.005	0.787

The relationship between body lipid and blood volume was not significant ($P > 0.05$), but the inclusion of liveweight into the regression considerably improved the relationship, and the partial correlation coefficients between body lipid and blood volume ($r = 0.626$) and liveweight ($r = 0.588$) were highly significant ($P < 0.001$).

Blood volume was also poorly associated with the fat-free constituents of the body. The relationship with the fat-free mass was close ($r = 0.819$) but the accuracy of prediction was not high, the R.S.D. of 3.385 kg was 5.70% of the mean fat-free weight. The unexplained variability in the empty body was 35% and for crude protein was 33%. These results suggest that although the blood volume was significantly associated with the fat-free mass, a large proportion of the variability in blood volume remains unexplained (32.9%), after accounting for the fat-free weight.

A comparison of the R.S.D's of the regression equations between blood volume and the chemical components of the body, with those of the regressions of the chemical components on liveweight, indicate that blood volume determinations offer little, in terms of accuracy, in the prediction of body composition over the use of liveweight alone.

The regressions between the chemical body components and the individual constituents of the blood – the plasma and the red cells, showed the same order of association as the relationship between the chemical components and blood volume.

The prediction of the dissectible components of the body by blood volume estimates. In general, the relationships between the estimated blood volume and the dissectible components of the body (Table 9.18) were more variable than those described above.

Table 9.18

The prediction of the dissectible components of the body by blood volume estimates (n = 24)

Independent variable x_1 = Blood volume (litres), x_2 = Liveweight (kg)

Dependent variable (y) (kg)	Regression equation	R.S.D. (kg)	R
Dissectible fat-free mass	$y = 5.791\ x_1 + 18.999$	2.638	0.846
Dissectible fat	$y = 0.579\ x_2 - 3.948\ x_1 - 1.594$	2.639	0.664
Dissectible lean	$y = 4.170\ x_1 + 3.809$	2.020	0.830
Commercial carcass yield	$y = 3.692\ x_1 + 28.636$	3.325	0.626

The closest relationship was that between blood volume and the dissectible fat-free body mass (r = 0.846); the R.S.D. of the regression equation was 2.638 kg which was 4.27% of the mean DFFM. The relationship between the dissectible lean and blood volume was also fairly close (r = 0.830). There was less variability in this relationship than that

between the chemical fat-free mass and blood volume. It is possible that dissectible lean and dissectible fat-free body are more representative of the active protoplasmic mass than is the fat-free mass. The correlation between dissectible fat and blood volume was not significant ($r = 0.215$, $P > 0.05$) but the inclusion of liveweight into the regression improved the relationship ($R = 0.664$), and the partial correlation coefficients between liveweight and blood volume and the dissectible fat, were significant ($P < 0.001$). In all of the regression equations presented in Table 9.18, there were large R.S.D's suggesting that for the dissectible and chemical components of the body, blood volume determinations contributed little to the accuracy of prediction, over liveweight alone.

(b) The prediction of body composition by non-dilution techniques - I

External measurements

The mean values of the external dimensions measured on the 24 pigs are recorded in Table 9.19. The individual values are presented in Appendix Table 14.

Table 9.19

The mean values, standard deviations and range of external dimensions (n = 24)

	Measurement	Mean (cm)	Standard deviation (cm)	Range (cm)
1.	Jaw length	22.1	2.0	18.0 - 27.0
2.	Jaw width	17.3	1.1	15.5 - 19.5
3.	Shoulder height	60.2	3.1	52.5 - 66.5
4.	Body length	91.2	5.1	77.0 -100.0
5.	Forearm length	35.1	2.1	30.0 - 38.5
6.	Hind-leg length	26.8	1.8	21.5 - 29.0
7.	Neck circumference	73.3	3.8	67.5 - 85.0
8.	Chest girth	102.0	4.2	94.0 -110.0

Before computing regression equations between the weights of the various components of the body and values of each of the external measurements a correlation matrix was computed. From this matrix the highest correlations between the body components and the body measurements were selected, and the regression equations subsequently calculated.

For the extremely low correlations which were obtained between the body components and the external measurements, it was concluded that the extremely small range of values recorded was not of sufficient magnitude to reflect differences in body composition. For example, in Table 9.19 the standard deviation of the jaw width measurement was only 1.1 cm which was 6.3% of the mean value, the maximum range of values recorded for this measurement was only 4.0 cm. In many instances the low correlations could also be attributed to the errors of measurement. The distance between two pre-defined points on the surface of the animal body was liable to considerable fluctuations, depending on the amount of flesh cover, and on fat pigs the location of bone extremities was found to be very difficult.

The prediction of the chemical and dissectible components of the body by external linear measurements. The regression equations between various components of the body and some of the external dimensions of the body are shown in Table 9.20.

From the correlation matrix, the correlation coefficient between total body lipid and the circumference of the neck was found to be the highest ($r = 0.700$). The R.S.D. of the regression equation was 3.372 kg which was 15.9% of the mean lipid weight. Although this was a relatively poor relationship, it was a considerable improvement over that between the body lipid and liveweight, the R.S.D. of this regression equation being 4.693 kg.

The relationship between the dissectible fat and the neck circumference was also fairly close ($r = 0.700$). The R.S.D. of the

regression equation was 2.351 kg which was 10.9% of the mean dissectible fat weight.

Some of the dimensions measured on the live animal were relatively highly correlated with the fat-free components of the body, although the relationships with the body ash were surprisingly low (see Table 9.20). This is possibly because the dimensions of an individual bone are not directly related to the weight of that bone, or indeed to the total skeletal mass.

The regression equations presented in Table 9.20 indicate that the external dimensions of pigs of approximately the same body weight are of little value in predicting body composition.

Table 9.20

The prediction of the chemical and dissectible components of the body by external measurements (n = 23)

Dependent variable (y) (kg)	Regression equation	R.S.D. (kg)	R
Total body lipid	$y = 0.852\ x_1 - 41.036$	3.372	0.700
" " "	$y = 51.967 - 1.377\ x_2$	3.715	0.617
Total Crude Protein	$y = 0.338\ x_1 + 0.155$	1.037	0.579
Total Ash	$y = 0.072\ x_2 + 0.024\ x_5 - 1.084$	0.228	0.678
" "	$y = 0.090\ x_4 + 0.126$	0.249	0.563
" "	$y = 0.072\ x_3 + 0.009$	0.259	0.517
Fat-free mass	$y = 1.211\ x_3 + 0.490\ x_5 - 24.216$	4.144	0.719
Dissectible fat	$y = 0.594\ x_1 - 25.636$	2.351	0.700

x_1 = Neck circumference (cm)
x_2 = Jaw length (cm)
x_3 = Forearm length (cm)
x_4 = Hind leg length (cm)
X_5 = Liveweight (kg)

Ultrasonic measurements

The results obtained for the ultrasonic technique are presented in two parts. In the first part (a) The relationships between the individual ultrasonic readings made by each operator and the true value obtained after slaughter of the animal are given. In the second part (b) The relationships between the ultrasonic readings and the body fat are presented.

(a) The relationships between the individual ultrasonic readings and the true backfat measurements were based on the data from only 19 pigs. The remaining five pigs were not used in the computation of these relationships because of the lack of attendance of some of the operators at the required times.

The correlation coefficients computed between the individual ultrasonic measurements recorded for each operator and the true backfat measurements are given in Table 9.21. In Appendix Table 15 the mean values of the individual ultrasonic measurements recorded by the three operators are given, in addition to the carcass backfat measurements.

Table 9.21

Correlations between the ultrasonic measurements and the corresponding carcass backfat depths (n = 19)

Measurement	Operator 1	Operator 2	Operator 3
Shoulder	0.870	0.855	0.821
Midback	0.868	0.880	0.853
Loin	0.803	0.865	0.719
"C"	0.781	0.810	0.787
"K"	0.718	0.788	0.753

In Table 9.21 it can be seen that the relationships between each of the ultrasonic backfat measurements and the carcass backfat measurements were similar for each of the three operators.

This was a surprising result, because in choosing the three operators it was anticipated that there would be a gradation of skills in the use of the equipment.

The similarity of the correlations shown in Table 9.21 for each measurement was possibly due to the fact that for a few of the pigs, operator 1 took his readings prior to and in the presence of either operators 2 or 3, because of the lack of an independent recording system. Thus, it was possible that operators 2 or 3 were influenced in recording and interpreting their measurements, by acting as recorder for operator 1. The differences between the mean values of the ultrasonic readings for the three operators were not significant, and thus there was no justification in ranking the operators in terms of accuracy.

There are several sources of error in making ultrasonic measurements on live pigs. These were discussed extensively in Chapter 5, but acting as operator 3, the author gained first-hand knowledge in defining the most likely sources of error in recording and interpreting ultrasonic measurements.

During the course of the measurements each animal was confined to a cage and given some feed. This was done to restrict the movement of the animal which would otherwise have tended to expand and contract the layers of backfat. Some of the pigs were found to be extremely hairy, and therefore at the points of measurement, the hair was removed in order to facilitate a uniform contact of the probe with the skin surface. In the leaner animals the maximum area of contact of the probe with the skin was reduced because of skeletal extremities. This was especially evident at the shoulder where the scapulae were often found protruding very near to the skin. Conversely, in the fatter pigs, errors in the estimation of backfat

depth were possibly due to physical depression of the subcutaneous fat by the probe.

Possibly one of the major sources of variation in the relationships between ultrasonic measurements and weight of body fat was in the definition of the sites of measurement on the live animal. Also, the difference in the positions of the measurements between the live animal and their resulting carcasses may have been partly responsible for the relatively poor correlations between the ultrasonic and carcass measurements (see Table 9.21). This was particularly evident for the C and K fat measurements which were taken at defined points on the animal and on the carcass.

The interpretation of the oscilloscope display was another major source of error. In the lean pigs, there was a tendency to confuse the peaks arising from the skin layer and those due to the fat layers. In the fatter animals the depth of backfat to the J layer (see Fig. 6.4) was often interpreted as either the C or K fat depth. Operator errors due to the incorrect calibration of the instrument display from recording fat thicknesses which were not at right angles to the surface of the skin, and from recording fat depths which were not taken at the pre-defined sites were also implicated.

(b) The correlations which were obtained between the ultrasonic and true fat depth measurements indicated that there was little advantage in computing regressions between the ultrasonic measurement recorded by each operator, and the body components. The independent variable used in these regressions was the mean value of the measurements obtained by the three operators. The correlation coefficients between the ultrasonic readings taken at various points on the animal body, and the body lipid and dissectible fat are given in Table 9.22.

Table 9.22

Correlations between the ultrasonic measurements, carcass backfat depths and the total body lipid and dissectible fat (n = 24)

	Total body lipid		Dissectible fat	
	Ultrasonics* (1)	Carcass measure-ments (2)	Ultrasonics* (3)	Carcass measure-ments (4)
Shoulder	0.750	0.698	0.777	0.720
Midback	0.752	0.822	0.750	0.817
Loin	0.832	0.836	0.813	0.850
"C"	0.630	0.855	0.610	0.814
"K"	0.621	0.875	0.604	0.831
Total**	0.767	0.901	0.763	0.892

* mean of three operators but also includes mean values of two operators

** sum of either the ultrasonic or carcass fat depths

The correlations between the fat measurements taken on the chilled carcass and body lipid and dissectible fat are also given for comparison. Indeed, a comparison of the adjacent correlation coefficients in columns 1 and 2 indicates that there was a relatively larger variability in the relationships between each of the ultrasonic measurements and total body lipid. This was attributed mainly to the inaccurate measurement of the depth of fat at each site, by ultrasonics. In particular the relationships between the C and K ultrasonic fat depths and body lipid were relatively poor, and may have been due to the difficulty in locating the positions at which these measurements were taken. The superior relationships between the fat depth measurements at these two points, on the carcass

with body lipid and dissectible fat, substantiates the findings of previous workers (McMeekan, 1941; Hankins and Ellis, 1934) who have found that the depth of fat over the longissimus dorsi is highly correlated with carcass fatness.

In general, the fat depths measured on the chilled carcass were more closely related to the body lipid and dissectible fat than were the fat depths measured on the live animal by ultrasonics. However, the correlations between the shoulder fat measurements taken on the chilled carcass and body lipid and dissectible fat were lower than the correlations between the shoulder fat depth measured ultrasonically on the live animal and these two carcass components.

The prediction of body lipid and dissectible fat by ultrasonic measurements. The regression equations computed between each of the ultrasonic measurements and body lipid and dissectible fat are shown in Table 9.23.

Table 9.23

The prediction of body lipid and dissectible fat from ultrasonic measurements* (n = 24)

Dependent variable y_1 = body lipid (kg), y_2 = dissectible fat (kg)

Dependent variable	Regression equation	R.S.D. (kg)	r
y_1	$y_1 = 0.571\ x_1 - 3.734$	3.155	0.750
y_1	$y_1 = 0.689\ x_2 + 8.696$	3.142	0.752
y_1	$y_1 = 0.764\ x_3 + 5.148$	2.654	0.831
y_1	$y_1 = 0.630\ x_4 + 10.946$	3.704	0.630
y_1	$y_1 = 0.603\ x_5 + 10.206$	3.740	0.621
y_2	$y_2 = 0.428\ x_1 - 0.992$	2.168	0.777
y_2	$y_2 = 0.496\ x_2 + 8.686$	2.281	0.750
y_2	$y_2 = 0.541\ x_3 + 6.338$	2.006	0.813
y_2	$y_2 = 0.441\ x_4 + 10.519$	2.732	0.610
y_2	$y_2 = 0.425\ x_5 + 9.958$	2.747	0.604

Independent variables:

x_1 = maximum shoulder fat depth (mm)
x_2 = minimum mid-back fat depth (mm)
x_3 = minimum loin fat depth (mm)
x_4 = "C" (mm)
x_5 = "K" (mm)
* = mean of six readings (2 readings x 3 operators)

There was found to be no increase in the accuracy of prediction of these equations by including liveweight as an independent variable. By comparing the R.S.D's of the regressions of body lipid and dissectible fat on the various backfat measurements, it was obvious that the measurements recorded on the dorsal midline accounted for more of the variability in the predicted variables than did the C and K measurements. There was also a trend for the measurements taken on the more posterior parts of the back of the animal to be more accurate in their prediction of body lipid and dissectible fat. The loin fat depth was the most accurate predictor of body lipid and dissectible fat. The RSD's of the regressions of these two body components on ultrasonic loin fat depth were 2.654 kg of body lipid and 2.006 kg of dissectible fat which represented 12.50% and 11.32%, respectively, of the mean values of these components.

Visual appraisal

The results obtained for the visual appraisal of each of the 24 animal are presented in two parts. In the first part (a) the individual judges' scores are presented and comparisons are made with the mean panel scores and the scores recorded for the other judges. In the second part (b) the regressions, computed between the body components and the mean visual assessment panel (VAP), are given.

(a) The individual scores recorded for each judge at each meeting of the VAP are recorded in Appendix Table 16. There were missing values throughout. This was due to the inability of some operators to attend these meetings at the stated times. Before commencing in any detail about the individual judges' scores, it is necessary to record the opinion of all the judges, that a seven-point scale would have been preferred to the five-point scale adopted here. This opinion was expressed only half-way through the experiment when the

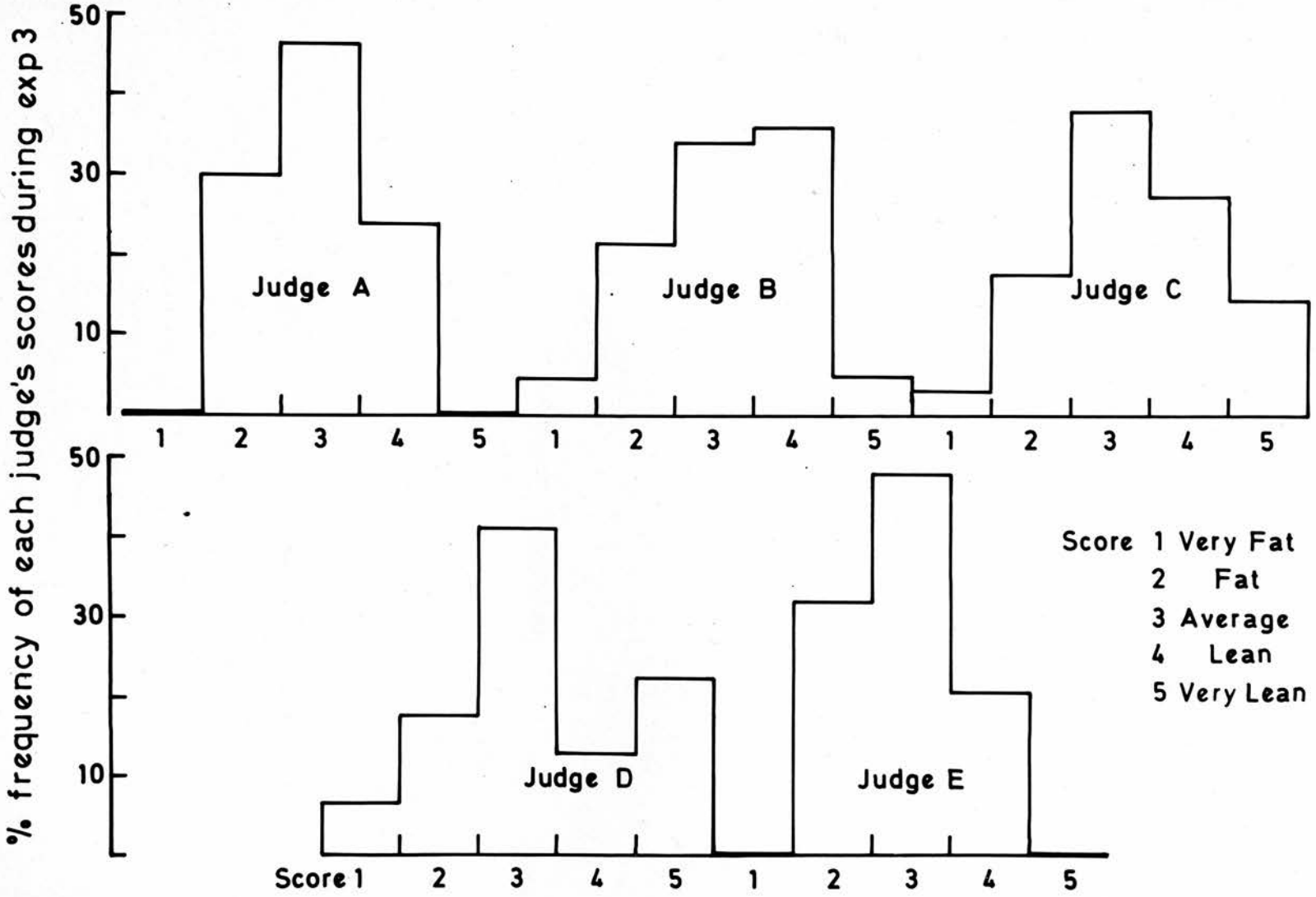


Fig. 9.7 Frequency Histograms For Each of the Judge's Scores in the Visual Assessment Panel.

leaner pigs were being assessed. It was concluded that the experience in assessing the fatness of the pigs had increased during the course of the experiment. However, the seven-point scale was not adopted because of the difficulties which were anticipated in the analysis of the data.

The mean assessment scores calculated for each judge are recorded in Table 9.24. It was found that judges C and D tended to exaggerate the fatness of the pigs while judges A and E tended to score the pigs on the leaner side of average. Judge B slightly over-estimated the fatness of the pigs, but his mean score did not differ significantly from those of judges A, C, D and E.

Table 9.24

The mean scores of each judge on the visual assessment panel

Judge	Mean overall score*	Standard deviation	Standard error of mean
A	2.9	0.74	0.09
B	3.1	0.97	0.12
C	3.3	1.02	0.12
D	3.3	1.18	0.15
E	2.9	0.72	0.09

Average score = 3.00

* Calculated from the scores of 72 pigs

Further analysis of the data indicated that judges A and E were modest in their scoring, because no extreme scores of Very Fat or Very Lean, were recorded. The standard errors of their mean scores were small in comparison to those of the other judges. Conversely, judge D was found to be more adventurous in his assessment of the animals, the extreme scores of Very Fat and Very Lean were recorded frequently. The frequency histograms of the scores of each judge are shown in Fig. 9.7.

(b) In this section, the scores recorded by each judge were not

considered in isolation, but a mean panel test score was computed for each pig, and provided the basis for computing the regression equations between the visual appraisal scores and body fatness. The experiment was not designed for testing the repeatability of each judge's assessment ability and thus there was no foundation for considering the opinion of any one judge to be more superior than the others, in estimating the fatness of the pigs.

The regressions in Table 9.25 indicate that, using the mean test panel score, the total body lipid in the live animal could be estimated to ± 2.511 kg which was 12.02% of the mean body lipid, and to ± 2.003 kg of dissectible fat which was 11.31% of the mean value. These equations do not apply to the results of other data, because of the specificity in the range of fatness from which these regressions were calculated. For pigs which are considerably fatter than the fattest pigs on this experiment, considerable errors would result in the prediction of body fat.

The inclusion of liveweight as an independent variable into these equations did not improve the accuracy of prediction of either body lipid or dissectible fat. Indeed, the partial correlation coefficients between liveweight and body lipid and dissectible fat were not significant at the 5% level.

Table 9.25

The prediction of total body lipid and dissectible fat by visual appraisal of the live animal (n = 23)

Independent variable (x) = Mean Test Panel Score

Dependent variable y (kg)	Regression equation	R.S.D. (kg)	r
Total body lipid	$y = 42.739\,x - 6.690$	2.551	0.844
Dissectible fat	$y = 32.608\,x - 4.626$	2.003	0.810

(c) The prediction of body composition by non-dilution techniques - II

Feed Conversion Ratio

The performance characteristics of the pigs. On the low protein diet (13.49% CP) the average daily liveweight gain of the castrates was significantly higher ($P < 0.001$) than that of the females (Table 9.26). On the high protein diet (22.08% CP) the differences in the liveweight gain between the castrates and females were not significant ($P > 0.05$).

The mean feed conversion ratios (F.C.R.) defined as kg of feed intake/kg of liveweight gain are also given in Table 9.26. It was found that there were no significant differences in F.C.R. between the castrates and females on each diet, but on the high protein diet there was a trend for the castrates to have slightly poorer F.C.R's.

The percentage lipid in the empty body of each of the pigs on the different treatments is also shown in Table 9.26. Although there were no significant differences ($P > 0.05$) in the proportions of body lipid between the castrates and females at each protein level, there was a trend for the castrates to be fatter than the females.

The prediction of body composition by F.C.R. and C.F.C.R. The individual values for the corrected feed conversion ratio (C.F.C.R.) and feed conversion ratio (F.C.R.) are given in Appendix Table 3.

The computed regression equations between the various body components and F.C.R. were found to be non-significant ($P > 0.05$) which indicates that F.C.R. is not a reliable indicator of the calorific density of the body.

By referring to Appendix Table 3 and Table 9.26 it can be seen that on the lower feed intakes pigs had slower growth rates which generally resulted in leaner carcasses having a lower energy per unit weight. However, the expected improvement in feed conversion ratio was offset by

Table 9.26

The performance data of the pigs in experiment 3 (n = 24)

Diet	% Protein ø	Sex	Performance Parameters	Dietary intake (g/kg $W^{0.75}$ ‡)					
				70	84	98	112	126	140
X	13.49	M	‡ Daily liveweight gain	0.29	0.42	0.42	0.53	0.54	0.71
			+ F.C.R.	3.96	3.61	3.64	3.41	3.58	3.21
			* Percentage body lipid	23.40	31.96	28.15	31.81	32.10	34.45
		F	‡ Daily liveweight gain	0.25	0.38	0.43	0.51	0.56	0.69
			+ F.C.R.	4.22	3.72	3.81	3.46	3.50	3.26
			* Percentage body lipid	23.17	26.95	31.28	29.93	33.78	33.94
Y	22.08	M	‡ Daily liveweight gain	0.29	0.37	0.52	0.58	0.58	0.64
			+ F.C.R.	3.77	3.71	2.91	3.23	3.03	3.43
			* Percentage body lipid	17.05	26.07	21.65	27.61	24.11	31.73
		F	‡ Daily liveweight gain	0.28	0.46	0.52	0.62	0.66	0.77
			+ F.C.R.	4.02	3.03	2.71	2.75	2.76	2.77
			* Percentage body lipid	17.73	19.73	19.83	19.55	20.94	25.30

‡ metabolic liveweight
ø air-dry basis
‡ kg
+ kg feed intake/kg liveweight gain
* in the empty body

the increase in the overall energy for maintenance because the slower growing pigs took longer to reach their slaughter weights.

The relationships between several of the body components and C.F.C.R. were found to be very close. In particular, body lipid and fat-free weight were highly correlated with C.F.C.R. which indicates that the calorific density of the carcass is closely associated with feed intake when adjustments are made for the overall energy requirements for maintenance. These relationships were considerably improved when live-weight was included as an independent variable. It is possible that the differences in liveweight at slaughter may have obscured the normally close relationships between these variables.

The correlation between C.F.C.R. and total carcass energy was high (R = 0.960) and the R.S.D. of the multiple regression was $\pm$ 12.29 kcal which was 4.64% of the mean value (see Table 9.27). C.F.C.R. was also highly correlated with the total body lipid (R = 0.953), the R.S.D. of the regression equation was $\pm$ 1.473 kg of lipid which was 6.94% of the mean lipid weight.

Table 9.27

The prediction of the chemical components of the body by the corrected feed conversion ratio (n = 24)

Independent variable (x_1) = corrected feed conversion ratio
(x_2) = Liveweight (kg)

Dependent variable y (kg)	Regression equation	R.S.D. (kg)	R
Total body water	$y = 0.368\ x_2 - 9.659\ x_1 + 34.553$	1.418	0.949
Total body lipid	$y = 13.584\ x_1 + 0.377\ x_2 - 39.225$	1.473	0.953
Total crude protein	$y = 0.117\ x_2 - 2.466\ x_1 + 7.375$	0.646	0.877
Fat-free mass	$y = 0.511\ x_2 - 12.622\ x_1 + 43.274$	2.007	0.943
Fat-free dry matter	$y = 0.144\ x_2 - 2.961\ x_1 + 8.714$	0.863	0.855
Total carcass energy (Kcal)	$y = 119.01\ x_1 + 4.274\ x_2 - 346.03$	12.293 (Kcal)	0.960

The correlations between C.F.C.R., liveweight and the fat-free components of the body were relatively high. C.F.C.R. in combination with liveweight was closely correlated with empty body water (R = 0.949) and the fat-free weight (R = 0.943), but the correlation with the weight of crude protein was not as high (R = 0.877).

The relationships between C.F.C.R. and the dissectible components of the body were also close, (see Table 9.28). The correlation between C.F.C.R., liveweight and dissectible fat was 0.963, and the R.S.D. of the regression equation was 0.947 kg which was 5.35% of the mean value.

Dissectible lean was not accurately predicted from C.F.C.R. even when liveweight was included in the regression equation. The multiple correlation was 0.848. The fact that the dissectible lean did not represent all of the lean deposits in the body possibly resulted in this relatively poor relationship.

The relationship between C.F.C.R., liveweight and the dissectible fat-free mass was closer (R = 0.952) than that with the chemically-defined fat-free mass (r = 0.943). The R.S.D. of the regression was 1.550 kg which was 2.51% of the mean value.

Table 9.28

The prediction of the dissectible components of the body by the corrected feed conversion ratio (n = 24)

Independent variable (x_1) = C.F.C.R.

(x_2) = Liveweight (kg)

Dependent variable (y) (kg)	Regression equation	R.S.D. (kg)	R
Dissectible fat-free mass	$y = 0.535\ x_2 - 9.335\ x_1 + 36.768$	1.550	0.952
Dissectible lean	$y = 0.373\ x_2 - 5.757\ x_1 + 15.611$	1.965	0.848
Dissectible fat	$y = 9.597\ x_1 + 0.349\ x_2 - 31.946$	0.947	0.963

Specific gravity

Carcass specific gravity was determined on each of the 24 pigs. The individual values are given in Appendix Table 17.

The average water temperature throughout the experiment was 12.5°C (S.D. = ± 2.55°C). No corrections were made for variation in the temperature as preliminary calculations had shown that fairly large differences in water temperature were required to produce measurable differences in the specific gravity of the carcass.

The prediction of body composition by carcass specific gravity. The relationships between the body components and the specific gravity of the left side of the carcass were close.

For all of the relationships the inclusion of liveweight into the regression equations as an independent variable significantly improved the accuracy of prediction. Differences in the liveweight of the animals, reflected in the range of carcass weights, presumably obscured the normally close inverse relationship which exists between the proportions of lean and fat in the body.

A further improvement in the accuracy of prediction was achieved by using empty body weight as an independent variable, instead of liveweight. The prediction of the chemical components from specific gravity and empty body weight is shown in Table 9.29.

Table 9.29

The prediction of the chemical components of the body by carcass specific gravity (n = 24)

Independent variable (x_1) = specific gravity

(x_2) = Empty body weight (kg)

Dependent variable y (kg)	Regression equation	R.S.D. (kg)	R
Total body water	$y = 312.91x_1 + 0.556x_2 - 327.60$	1.166	0.966
Total body lipid	$y = 0.227x_2 - 417.85x_1 + 440.49$	1.286	0.965
Total crude protein	$y = 84.116x_1 + 0.176x_2 - 90.278$	0.486	0.932
Fat-free mass	$y = 417.85x_1 + 0.773x_2 - 440.49$	1.286	0.977
Fat-free dry matter	$y = 104.84x_1 + 0.217x_2 - 112.79$	0.603	0.932

The regressions of the dissectible components of the body on carcass specific gravity are presented in Table 9.30.

Table 9.30

The prediction of the dissectible components of the body by carcass specific gravity (n = 24)

Independent variable (x_1) = Specific gravity
(x_2) = Empty body weight (kg)

Dependent variable y (kg)	Regression equation	R.S.D. (kg)	R
Dissectible fat-free mass	$y = 304.01x_1 + 0.746x_2 - 316.69$	1.263	0.968
Dissectible fat	$y = 0.258x_2 - 283.58x_1 + 293.87$	1.125	0.947
Dissectible lean	$y = 193.27x_1 + 0.524x_2 - 210.03$	1.706	0.888

It can be seen from this table that the relationship between specific gravity, empty body weight and the weight of dissectible fat was close (R = 0.945). The R.S.D. of the regression was 1.125 kg which was 6.35% of the mean value. Specific gravity in combination with empty body weight was also closely related to the weight of dissectible lean (R = 0.888), the R.S.D. of the regression being 4.92% of the mean dissectible lean weight. Dissectible fat-free mass was highly correlated with specific gravity and empty body weight (R = 0.968), the R.S.D. of this regression being 1.263 kg which was only 2.04% of the mean value.

Although the relationships between specific gravity and the chemical and dissectible body components were close, the unexplained variability indicates that either a true inverse relationship between the fat and lean did not exist, or that there was a variation in the density of these constituents.

It was also possible that variation in the density of the bone in the carcass could upset the simple relationships which exist between

specific gravity and the body components. However, a statistical analysis of the data showed that lean/fat ratio was more highly correlated with specific gravity ($r = 0.903$) than was the lean/bone ratio ($r = 0.224$). This clearly demonstrates that the specific gravity of the carcass was more closely associated with the relative proportions of fat and lean in the carcass than the proportions of dissectible bone and lean. This trend was still evident when the data were standardised according to empty body weight.

Discussion and Conclusions

The experiment has shown in a convincing manner the relative accuracy of several different types of indirect technique for estimating *in vivo* composition in the bacon pig.

The regression equations which were computed between the body components and the various predictors has allowed comparisons to be made, not only between the indirect methods, but also with the regressions of the body constituents on liveweight. Liveweight at slaughter accounted for a considerable proportion of the variability in the fat-free constituents of the body. Over a range of body weights, the predictive efficiency of any indirect technique can be assessed by comparing it with the predictive accuracy of the best single index, liveweight.

From the results obtained in this experiment, it is clear that the indirect techniques can be ranked according to their accuracy of prediction. Generally, it was found that the most expensive technique gave the most accurate assessment of body composition.

The non-dilution techniques such as ultrasonics, visual appraisal and the measurement of the body dimensions did not give relatively accurate

estimates of the body components, However, the advantages of such techniques are that they are simple and inexpensive to operate and obviously are of use in circumstances in which the more sophisticated techniques cannot be applied.

Visual appraisal was possibly the cheapest and simplest of all the indirect techniques applied in this experiment. The assessment of body composition by a subjective technique such as this, is not very accurate, but this must be offset against its simplicity. The measurement of the external dimensions of the body can be considered as the simplest quantitative technique for assessing body composition. From the results of this experiment it can be seen that external body measurements also gave relatively inaccurate estimates of the amounts of either the chemical or dissectible body components.

There are several possible sources of error in the technique, such as errors in the measurement of the length of bones, difficulties in locating anatomical points and differences in subcutaneous fat cover.

In spite of these general criticisms of the method, certain measurements such as neck circumference and jaw-length provided a more accurate assessment of some body components than did liveweight.

The ultrasonic technique was the only one of these non-dilution methods to provide details of some internal dimensions of the body. The potential accuracy of the method was possibly not realised because of the difficulty in locating the positions at which the depth of subcutaneous fat is known to be highly associated with the amount of dissectible fat. In particular, the C and K measurements, which were spot locations, were only poorly correlated ($r = 0.610$ and 0.604, respectively) with the weight of dissectible fat. In contrast, the ultrasonic measurements taken along the mid-dorsal line were more accurate in predicting the weight of dissectible fat.

A reverse situation was found when similar measurements were taken on the chilled carcass. The C and K measurements on the carcass were more closely related to the weight of dissectible fat (r = 0.814 and 0.831, respectively) than were the measurements taken on the mid-dorsal line.

It would therefore seem that there is an obvious requirement for experienced operators who can (a) define the correct positions at which the measurements should be made, (b) accurately interpret the oscilloscope display.

In this experiment it was found that there were only small differences in the precision of the three operators in measuring backfat thickness by ultrasonics. However, the pigs in this experiment could not be considered representative of a commercial population in which extremes of fatness are not commonly found, and in a population of leaner animals the differences in precision between these operators may have been more marked. However it would possibly be difficult to assess the accuracy the ultrasonic technique in a population of leaner animals. In such animals it is possible that a thinner subcutaneous fat cover would permit a more exact location of the sites of measurement, but it is also possible that errors in the interpretation of the oscilloscope display would be increased. From the work of Stouffer et al. (1961) it would seem that larger errors result in the interpretation of the oscilloscope display when there is a relatively smaller depth of backfat.

The first information on the size of the fat-free components of the body was provided by the dilution techniques, ^{42}K, D_2O and Evans Blue. The ^{42}K technique was the most expensive of these three but provided the

most accurate estimates of the fat-free constituents of the body. Conversely the Evans Blue dilution technique was the simplest and cheapest of these three methods, and provided the least accurate estimates of the fat-free components.

The D_2O dilution technique, which was used for estimating the body water space, gave relatively accurate estimates of the fat-free components of the body, but the potential accuracy of the method was possibly not realised. Analysis of the results showed that D_2O estimated TBW more closely than EBW. TBW included not only the water in the empty body, but also that of the intestinal tract which can be quite variable. Further investigation of this technique could be directed towards determining the rates of transfer of D_2O across the gut-wall, with the objective of finding the optimum time of sampling, so that an estimation of the EBW could be made.

From the results it was also found that D_2O overestimated the TBW space, which is in agreement with previous studies (Krogh and Ussing, 1936; Ussing, 1938) in which it has been suggested that D_2O also exchanges with the more labile hydrogen ions in fat and protein. In the present study, D_2O space was found to be, on average, 2.1% larger than the TBW space, although this figure included the results from three pigs in which there was an underestimate of TBW space.

Although it has been shown in this experiment that the D_2O dilution technique can provide relatively accurate estimates of body composition, it is possible that the method would need certain modifications if it were to be applied in routine practical situations. Further research could be directed towards investigating the effects of the type and quantity of feed on gut-fill, the effects of administering D_2O by other routes, and the development of simpler methods of analysis.

The Evans Blue technique for estimating blood volume was found to be the least promising of the three dilution methods. The results obtained in this experiment indicated that, although the estimated blood volume was more closely associated with the fat-free body weight ($r = 0.819$) than with liveweight ($r = 0.698$), the estimate of the weight of fat-free material in the live animal by this method was not very accurate.

The estimates of blood volume obtained in this experiment were higher than those obtained in previous studies (Bush et al., 1955; Hansard, Sauberlich and Comar, 1951; Doornenbal, Asdell and Wellington, 1962) and it is suggested that differences in the procedure of obtaining the blood samples were mainly responsible for this discrepancy. Whereas in previous studies blood volume was estimated in conscious animals by labelling the red cells with radioactive markers, the animals in the present experiment were probably still under the influence of anaesthetic when the plasma was labelled with the dye, Evans Blue.

The close relationship which was fouhd between blood volume and dissectible lean ($r = 0.830$) suggests that the muscle-mass of the body is more indicative of the active protoplasmic mass than is the fat-free mass. Indeed, the relationship between the fat-free, mineral-free body mass and blood volume was found to be higher ($r = 0.830$) than the relationship between blood volume and fat-free weight ($r = 0.819$).

In considering the Evans Blue dilution technique in terms of practical application, it is doubtful if the method offers much above that provided by simpler methods, such as ultrasonics. Although the Evans Blue technique is inexpensive in terms of materials and labour, the fairly rigid conditions under which it must be applied, and the lack of direct verification of the method, lends doubt as to the usefulness of the method for predicting in vivo body composition.

The C.F.C.R. and specific gravity methods gave remarkably accurate estimates of body composition despite the fact that they were simple and inexpensive to implement. They can be considered as differential methods, because their values depend on the relative proportions of two body components, fat and fat-free material.

C.F.C.R. provided extremely accurate estimates of body composition in the live animal, despite the assumptions which were made in the calculations. The potential accuracy of the method may not have been fully realised, because the values were calculated on a liveweight basis, and not on empty body weight.

In practical terms, the method has much to offer because it is simple and inexpensive, and as has been demonstrated in this experiment, gives remarkably accurate estimates of body composition. The method may need further investigation to determine its efficacy when different types of feed are used, under different types of feeding regime, when different genotypes are being used, when the values of C.F.C.R. are calculated over a smaller weight range than that used in this experiment.

Specific gravity measurements on the carcass also gave very accurate estimates of body composition. However, the determination of carcass specific gravity is restricted because it can only be made after the animal has been slaughtered. Nevertheless, from the results obtained in this experiment it is obvious that in certain situations, carcass specific gravity determinations can give accurate and immediate information on the composition of slaughtered pigs.

Although the results in this experiment have shown the relative accuracy of several indirect measurements of body composition, the data strictly apply to the pigs which were used in this study. Pigs of

different age, breed, strain and health, or pigs reared along different growth curves would almost certainly have a different body composition to the pigs used in this experiment. Indeed it is possible that even at constant liveweight, the composition of the fat-free body would not be constant over a wide range of fatness.

It is obvious that more studies are required in which the relationships between body components can be determined for different genotypes at different ages and different body weights. Such basic information is essential for the development and application of *in vivo* techniques for estimating body composition. Some of this information is already documented but it is of doubtful value because of the small number of animals from which it was derived. In work of this type it is difficult to assess the extent to which the variation in the composition of the fat-free body between animals is due either to errors in sampling procedure, or is biological variation. A more reliable assessment of the relative contribution of these two sources of variation could be provided by analysing a large number of animals of different sex, breed, age and weight.

CHAPTER 10

THE PREDICTION OF BODY COMPOSITION USING COMBINATIONS OF PREDICTORS

Introduction

It has been recognised for some considerable time that a more accurate assessment of body composition can be obtained by the simultaneous estimation of several body components, rather than the determination of one component. The limitations of predicting *in vivo* body composition by only one predictor were discussed in Chapter 4.

In the present study, several selected *in vivo* techniques were applied simultaneously to the same animal so that each predictor could then be compared with others in terms of accuracy, and also be combined with several other predictors, to obtain more information on the composition of the body. It is the latter objective which is dealt with in this chapter.

Re-synthesis of the data

The results obtained for each predictor, in experiment 3, were re-synthesised in the manner described below.

The numerous predictors of body composition were subjected to statistical analysis which (a) gave the total correlation coefficients between the predictors and the body components (b) adjusted the total correlation coefficient when one of the predictors (x_1) was kept constant, so that the next largest partial correlation coefficient could be determined, (c) repeated process (b) with the predictor (x_2) which had the largest correlation coefficient also kept constant, (d) repeated process (c) till the remaining partial correlation coefficients between the body

components and the predictors became non-significant at the 5% level.

From this analysis of the data, combinations of predictors were obtained which accounted for most of the variability in each body component. The prediction equations were obtained by applying multiple regression analysis to these combinations of predictors.

Results and Discussion

The prediction equations of several body components using the most accurate combinations of predictors are shown in Table 10.1. Inspection of these equations reveals that remarkably accurate estimates of the body components were made, using combinations of several predictors. The accuracy of each of these equations can be compared with equations in which only single predictors were used. For example, body lipid can be predicted to $\pm$ 0.850 kg when four different *in vivo* measurements are made. This is a much more accurate estimate than that obtained by using C.F.C.R. alone (R.S.D. = 1.473 kg). This substantiates the thesis that the composition of the body of the live animal can be more accurately determined when several predictors are used in combination, than by using only one predictor.

In a number of the equations shown in Table 10.1 it was found that the partial correlation coefficients between C.F.C.R. and the body components were the largest, indicating that this predictor accounted for most of the variability in the body components. C.F.C.R. is a differential type of measurement, its value depending on the relative proportions of fat and fat-free material in the body. It is assumed therefore, that the other predictors in the regression equations provided supplementary information on the variation in the fat-free material and body lipid which C.F.C.R. alone could not explain.

Table 10.1

The prediction of body components, using the most accurate combination of predictors (n = 21)

Dependent Variable (y) (kg)	Regression equation	R.S.D. (kg)	R
Total body water	$y = 0.169x_1 + 0.143x_2 - 0.098x_4 + 20.718$	0.642	0.992
Total body lipid	$y = 0.553x_1 - 0.151x_2 + 7.791x_3 + 0.130x_4 - 21.057$	0.850	0.987
Total crude protein	$y = 0.083x_2 - 0.432$	0.455	0.920
Fat-free mass	$y = 0.155x_1 + 0.224x_2 - 6.742x_3 + 26.244$	0.889	0.990
Dissectible fat	$y = 0.338x_1 + 8.329x_3 + 0.131x_5 - 33.976$	0.678	0.981
Dissectible lean	$y = 0.446x_1 - 7.449x_3 - 0.459x_6 + 40.695$	1.317	0.936

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Independent variables:
x_1 = Liveweight (kg)
x_2 = Potassium 42 space (g)
x_3 = C.F.C.R.
x_4 = Ultrasonic loin fat depth (mm)
x_5 = Ultrasonic shoulder fat depth (mm)
x_6 = Shoulder height (cm)

It can be seen from this table that potassium 42 space in isolation accounted for most of the variability in the weight of crude protein in the body. The inclusion of the other predictors into the regression equation did not improve the accuracy of estimate of crude protein. It is pertinent to note that the weight of crude protein was only relatively poorly associated with other major components of the body such as body water.

In the remainder of this chapter, examples are given to show how the use of certain combinations of predictors can reduce the errors in the prediction of certain body components.

For the prediction of fat-free mass in the live animal many of the indirect techniques rely on the general assumption that there are constant relationships between the constituents of the fat-free body. It would therefore seem possible to accurately predict the fat-free weight from a determination of either body water or body potassium. In practice, neither body water nor body potassium bear a constant relation with the fat-free body and thus relatively large errors in its estimation can result.

In equations 1 and 2, given in Table 10.2, it can be seen that the relationships between potassium 42 space (K_e), deuterium oxide space (D_2O) and fat-free weight were extremely close.

Table 10.2

The prediction of fat-free weight and body lipid by predictors used singly or in combination (n = 22)

Dependent variables y_1 = Fat-free wt (kg) y_2 = wt of body lipid (kg)

Regression equation	R.S.D. (kg)	R
1) $y_1 = 0.405\ K_e - 3.210$	1.347	0.976
2) $y_1 = 1.149\ D_2O + 3.470$	1.876	0.948
3) $y_1 = 0.270\ K_e + 0.388\ D_2O - 1.094$	1.192	0.982
4) $y_1 = 0.199$ L.wt. $+ 0.331\ K_e - 0.153$ S.F.US $- 1.777$	1.080	0.986
5) $y_2 = 16.483 - 0.383\ K_e + 0.762$ L.wt.	1.599	0.950
6) $y_2 = 4.759 - 1.216\ D_2O + 0.904$ L.wt.	1.420	0.957
7) $y_2 = 0.900$ L.wt. $- 0.162\ K_e - 0.761\ D_2O + 7.728$	1.279	0.968

Independent variables

L.wt. = Liveweight (kg)
K_e = Potassium determined by potassium 42 dilution
D_2O = Body water determined by deuterium oxide dilution (mm)
S.F.US = Maximum shoulder fat determined by ultrasonics (mm)

The accuracy of prediction of the fat-free weight was substantially increased when D_2O and ^{42}K determinations were made simultaneously (equation 3). The R.S.D. of this regression was much lower than those R.S.D's in either equations 1 or 2. This indicates that when two different assessments of the same component are made, then a more accurate estimate of the size of that component can be achieved. Indeed, the accuracy of prediction of the fat-free weight was increased when some measurement of body fat (shoulder fat depth) was made in conjunction with a determination of potassium space (see equation 4). The multiple correlation coefficient between the shoulder fat depth, ^{42}K space and the fat-free weight was 0.986 and the R.S.D. of the regression was 1.080 kg.

Similarly, it was found that although potassium 42 and D_2O dilution each gave fairly accurate estimates of body lipid (see equations 5 and 6, Table 10.2), in combination these two predictors gave a much more accurate prediction (see equation 7).

It would seem from this treatment of the data that the accuracy of the estimates of body components can be substantially improved when certain predictors are used in combination. To illustrate this in a more compact manner, the relative predictive efficiencies of several predictors used singly or in combination for estimating body lipid are given in the following table.

Table 10.3

The relative predictive efficiencies of several predictors used singly or in combination, for estimating the weight of body lipid (R.S.D. of D_2O estimate = 100%)

Predictors	Relative Predictive Efficiency (%)
(a) Single	
D_2O	100.0
C.F.C.R.	96.4
^{42}K	88.8
Visual Assessment Score	55.7
Loin fat depth	53.5
Neck circumference	42.1
Evans Blue	37.9
(b) Combinations	
^{42}K, C.F.C.R. and loin fat depth	167.1
^{42}K, D_2O	111.1

Conclusions

The estimation of several components of the body was found to be more accurate when predictors were used in combination, than when they were used in isolation.

From the equations obtained for the prediction of fat-free weight and body lipid, it seems that the use of combinations of predictors which give information on the size of the same component (e.g. ^{42}K and D_2O) can give an increase in the accuracy of prediction of that component, over the use of each predictor in isolation. It would also seem possible that further increases in the accuracy of prediction of a body component can be achieved by combining predictors which measure different components.

CHAPTER 11

THE OPTIMISATION OF COMBINATIONS OF PREDICTORS IN SPECIFIC SITUATIONS RELATED TO ANIMAL PRODUCTION

Introduction

In Chapter 10 it was found that combinations of certain predictors gave extremely accurate estimates of several body components. This was an ideal situation in which there was no restraint on the type of indirect technique which could be applied to the animal. However, in some practical situations the lack of facilities and equipment may preclude the measurement of some of the components in the live animal. In these circumstances the only alternative is to use a less expensive or less sophisticated technique, and this may not give the required degree of accuracy.

From the results which were obtained in the previous chapter it would seem possible that, in such circumstances, two or more of these simpler methods in combination could predict body composition with a similar degree of accuracy as one sophisticated technique used in isolation. In this chapter a number of multiple regression equations are given for the prediction of body composition in four situations related to animal production.

Re-synthesis of the data

The data obtained for the predictors described in Chapter 9, were re-synthesised in the manner described in Chapter 10. In addition two correlation matrices were compiled (see Appendix Tables 18a and 18b). These show (a) the correlations between all the predictors and all the

body components, (b) the correlations between all the predictors.

From these matrices it is possible to select any combination of predictors and calculate the multiple correlation coefficient with any of the body components. It is therefore possible to obtain an estimate of the variation in any of the predicted variables, which can be explained by combinations of predictors.

Results and Discussion

The four situations related to animal production were shown in Fig. 5.1 and were considered to cover a wide range of circumstances in which the knowledge of body composition is required. In each of these situations it was considered that total body lipid, fat-free mass, dissectible fat and dissectible lean would adequately describe the composition of the animal in both chemical and physical terms.

Example 1. The prediction of body composition in fundamental research situations. At this level, it was considered that a knowledge of body composition would be required in two distinct situations, (a) in situations in which sequential changes in body composition were being followed, (b) in situations in which the body composition at the end of periods of growth was required.

In both of these situations it was considered that the equipment and the facilities for making the in vivo measurements would be similar, except that in situation (b) in addition to facilities for the handling and analysis of radioactive and non-radioactive tracers, there was individual feeding accommodation, so that a measurement of the corrected feed conversion ratio could be made.

In the first situation, the following indirect methods were considered:- ^{42}K, D_2O and Evans Blue dilution, ultrasonics, external measurements and visual appraisal. The multiple regression equations

between the combinations of predictors and the body components are shown in Table 11.1.A. It can be seen that although extremely accurate estimates of the body components were made by these combinations of predictors, the accuracy of prediction of the dissectible components of the body was lower than that for the chemical components. This was possibly due to the heterogenous composition of the dissectible body components and also because they each represented only a proportion of the chemical component in the body.

In the second situation, combinations of ^{42}K space, C.F.C.R., ultrasonic loin-fat depth and liveweight were found to account for most of the variability in the body components. The multiple regression equations are shown in Table 11.1.B. From the partial correlation coefficients in each of these equations, it was found that C.F.C.R. accounted for most of the variability in each of the body components. It is concluded that the other predictors in the equations, provided supplementary information on the size of the components which C.F.C.R. alone could not explain.

For the prediction of dissectible lean it was found that a combination of liveweight and C.F.C.R. accounted for most of the variability in this component, and that the inclusion of other predictors into the prediction equation did not substantially improve the accuracy of estimate.

Example 2. The prediction of body composition in large-scale testing schemes. The scale of enterprise and the facilities which are available in many large performance-testing schemes often preclude the use of a number of methods for estimating body composition which involve time-consuming analytical procedures or expensive tracer substances.

For performance-testing schemes it was considered that measurements of the feed conversion ratio adjusted for maintenance (C.F.C.R.),

Table 11.1.A

The prediction of body components in fundamental research situations - A ($n \doteq 22$)

Dependent Variable y (kg)	Regression equation	R.S.D. (kg)	R
Total body lipid	$y = 0.900$ L.wt. $- 0.162\ K_e - 0.761\ D_2O + 7.728$	1.279	0.968
Fat-free mass	$y = 0.199$ L.wt. $+ 0.331\ K_e - 0.153$ S.F.US $- 1.777$	1.080	0.986
Dissectible fat	$y = 0.401$ L.wt. $- 0.174\ K_e + 0.208$ L.F.US $+ 6.687$	1.407	0.916
Dissectible lean	$y = 0.366$ L.wt. $+ 0.178\ K_e - 0.450$ Sh.Ht. $+ 3.391$	1.640	0.899

L.wt. = Liveweight (kg)
K_e = Potassium determined by ^{42}K dilution (g)
D_2O = Body water determined by D_2O dilution (kg)
S.F.US = Maximum shoulder fat measured by ultrasonics (mm)
L.F.US = Minimum loin fat measured by ultrasonics (mm)
Sh.Ht. = Shoulder height (cm)

Table 11.1.B

The prediction of body components in fundamental research situations - B ($n = 22$)

Dependent variable y (kg)	Regression equations	R.S.D. (kg)	R
Total body lipid	$y = 0.553$ L.wt. $- 0.151\ K_e + 7.791$ C.F.C.R. $+ 0.130$ L.F.US $- 21.057$	0.851	0.987
Fat-free mass	$y = 0.155$ L.wt. $+ 0.224\ K_e + 6.742$ C.F.C.R. $+ 22.244$	0.889	0.990
Dissectible fat	$y = 0.298$ L.wt. $+ 7.877$ C.F.C.R. $+ 0.141$ S.F.US $- 30.004$	0.707	0.981
Dissectible lean	$y = 0.446$ L.wt. $- 7.449$ C.F.C.R. $- 0.459$ Sh.Ht. $+ 40.695$	1.317	0.936

L.wt. = Liveweight (kg)
K_e = Potassium determined by ^{42}K dilution (g)
C.F.C.R. = Feed conversion ratio adjusted for maintenance
L.F.US = Minimum loin fat measured by ultrasonics (mm)
S.F.US = Maximum shoulder fat measured by ultrasonics (mm)
Sh.Ht. = Shoulder height (cm)

backfat depth by ultrasonics, external dimensions, and visual appraisal would provide sufficient information on the body composition of the animals on test. The multiple regression equations which were derived are shown in Table 11.2.

It can be seen from this Table that remarkably accurate estimates of each of the four body components were made, using these combinations of predictors. It was also found that the partial correlation coefficient between C.F.C.R. and the body components was the largest, indicating that this measurement accounted for most of the variability in these components. These prediction equations can be compared in terms of accuracy with those shown in Table 9.27 in which only C.F.C.R. and liveweight in combination were the predictors. It can be seen that for the prediction of body lipid, the simple expedient of measuring loin fat depth by ultrasonics, reduced the R.S.D. of body lipid from 1.473 kg to 1.297 kg, a reduction of nearly 12%. Similar increases in the accuracy of prediction of most of the other body components were also found when simple measurements were made in addition to the measurement of C.F.C.R.

Example 3. The prediction of body composition in situations in which there are limited technical resources. In a number of situations such as in research centres and in testing schemes, the body composition of animals at the end of nutritional regimes is often determined by either physical dissection or by chemical analysis of the carcass. Such procedures usually involve much expense in terms of labour and loss of saleable meat.

In these situations it is considered that combinations of several indirect measurements made on the split carcass of the animal could possibly give an accurate assessment of the composition of the animal without the necessity for detailed and laborious analytical procedures and without the loss of saleable meat.

The predictors which were considered were specific gravity and backfat measurements.

Table 11.2

The prediction of body components in large-scale testing schemes (n = 22)

Dependent variable y (kg)	Regression equation	R.S.D.(kg)	R
Total body lipid	y = 0.315 L.wt. + 10.901 C.F.C.R.+ 0.222 L.F.US - 32.980	1.297	0.966
Fat-free mass	y = 0.499 L.wt. - 11.069 C.F.C.R.+ 0.508 J.Len. + 29.759	1.826	0.955
Dissectible fat	y = 0.299 L.wt. + 8.190 C.F.C.R.+ 1.105 S.F.US - 29.580	0.821	0.974
Dissectible lean	y = 0.446 L.wt. - 7.449 C.F.C.R.- 0.459 Sh.Ht. + 40.695	1.317	0.936

L.wt. = Liveweight (kg)
C.F.C.R. = Feed conversion ratio adjusted for maintenance
L.F.US = Minimum depth of loin fat measured by ultrasonics (mm)
S.F.US = Maximum depth of shoulder fat measured by ultrasonics (mm)
J. Len. = Jaw length (cm)
Sh.Ht. = Shoulder height (cm)

Table 11.3

The prediction of body components in selection schemes in which there are limited technical resources (n = 24)

Dependent variable y (kg)	Regression equation	R.S.D.(kg)	R
Total body lipid	y = 0.186 E.wt. - 328.24 S.G. + 0.178 K + 346.43	1.114	0.975
Fat-free mass	y = 0.814 E.wt. + 328.24 S.G. - 0.178 K - 346.43	1.114	0.984
Dissectible fat	y = 0.258 E.wt. - 283.58 S.G. + 293.87	1.125	0.948
Dissectible lean	y = 0.524 E.wt. + 193.27 - 210.03	1.706	0.888

E.wt. = Empty body weight of the pig at slaughter (kg)
S.G. = Specific gravity of the left side of the carcass
K = K fat depth (mm)

The multiple regression equations which were derived indicated that most of the variability in the weights of the major components of the carcass could be explained by various combinations of empty body weight, specific gravity and the K fat depth. These are shown in Table 11.3.

In this table it can be seen that extremely accurate estimates of each of the body components were made and the chemically-defined components were predicted more accurately than were the dissectible components. In each of the regression equations it was found that the partial correlation between specific gravity and the predicted variable was the largest, indicating that this relatively simple measurement accounted for most of the variability in the body component. A comparison of the accuracy of these equations with those involving only specific gravity and empty body weight in combination (Table 9.29) shows that the inclusion of K fat depth into the regression considerably improved the accuracy of prediction. For the prediction of body lipid by specific gravity and empty body weight, the R.S.D. was 1.286 kg. In combination with the K fat depth, the R.S.D. was reduced by 13% to 1.114 kg. A similar increase in the accuracy of prediction of the fat-free mass was also found when these three measurements were made over the use of empty body weight and specific gravity.

Example 4. The prediction of body composition in non-technical situations. In a large number of commercial pig-breeding and rearing enterprises, a substantial proportion of the replacement stock is selected from those pigs intended for bacon production. Usually the selection of potential breeding stock is made by visual assessment which can sometimes result in pigs being selected on the basis of traits which are not related to either performance or carcass quality.

It is considered that in these circumstances the precision of a selection index for potential breeding stock could be increased by the

application of other simple *in vivo* measurements in addition to visual appraisal.

The methods which were considered were visual appraisal, ultrasonics and the measurement of various external dimensions. The combinations of these measurements which accounted for most of the variability in the body components are shown in Table 11.4.

It can be seen from Table 11.4 that the relationships between the predictors and the body components were generally not as precise as those obtained in the previous situations in which more sophisticated techniques were applied. However, a prominent feature of these results is that accurate estimates of the body components were made by combinations of extremely simple and inexpensive techniques. From these equations it can also be seen that the simple expedient of visually assessing an animal, and measuring one external body dimension, such as neck circumference, can give an estimate of body lipid with a similar degree of accuracy (R.S.D. = 2.040 kg) as the more sophisticated techniques such as ultrasonics and blood volume measurements in isolation (see Tables 9.23 and 9.17, respectively). Similarly, it was found that a combination of liveweight, loin fat depth and C fat depth measured by ultrasonics gave a more accurate estimate of the dissectible lean than did D_2O when it was used in isolation. For this combination of predictors the multiple correlation coefficient with dissectible lean was 0.834 and the R.S.D. of the regression equation was 2.008 kg. For D_2O the correlation with dissectible lean was 0.837 and the R.S.D. of the regression equation was 2.166 kg.

Table 11.4

The prediction of body composition in non-technical situations (n = 22)

Dependent Variable y (kg)	Regression equation	R.S.D. (kg)	R
Total body lipid	y = 0.215 L.wt. - 4.307 V.A.P. + 0.585 N.Cir. + 26.713	2.040	0.911
Fat-free mass	y = 0.947 L.wt. - 0.593 N.Cir. + 0.445 L.F.US + 32.542	2.377	0.925
Dissectible fat	y = 0.275 L.F.US - 2.494 V.A.P. + 20.136	1.727	0.854
Dissectible lean	y = 0.452 L.wt. - L.F.US + 0.440 C + 3.387	2.008	0.834

L.wt. = Liveweight (kg)
V.A.P. = Mean visual assessment panel score
N.Cir. = Neck circumference (cm)
L.F.US = Minimum loin fat depth measured by ultrasonics (mm)
C = C fat depth measured by ultrasonics (mm)

Conclusions

From the examples which were given in this chapter it is clear that in many situations related to animal production, combinations of several predictors can give extremely accurate estimates of body composition.

It can be expected that with a reduction in the available resources, the accuracy of estimate of body composition will decline. However, even with limited resources, certain combinations of inexpensive and simple measurements can predict several body components with the same degree of accuracy as some of the more sophisticated and expensive techniques.

It seems that even with the application of the most sophisticated techniques, a substantial improvement in accuracy of prediction can be achieved by also applying relatively simple measurements.

INTEGRATING DISCUSSION AND CONCLUSIONS

Introduction

It is the intention in this discussion to (a) emphasise the main conclusions obtained from the experimental section, (b) discuss the results of the investigation in the context of practical situations related to animal production and suggest possible areas of research in which further developments could be made.

(a) The main conclusions obtained from the experimental section

The results which were obtained in the experimental section will be considered in two parts. In the first part the relationships between the chemical components of the body will be discussed, and in the second part the results obtained for each of the indirect methods will be discussed.

(i) The data obtained from the chemical analyses of the pigs used in experiments 1 and 3 showed that although there was a large range in the body fatness, the composition of the fat-free body remained remarkably constant. It was found that the water content of the fat-free material of the 17 pigs used in experiment 1 was 74.14% (S.D. = $\pm$ 1.416%), and that of the fat-free material of the 24 pigs in experiment 3, 75.61% (S.D. = $\pm$0.955%). There were no significant differences ($P > 0.05$) between the castrates and females of experiment 3, but there was a significant difference ($P < 0.001$) between experiments. This was a surprising result because animals of similar breed, age and weight were used in both experiments. It is possible that the lower water/fat-free body ratio of the pigs in experiment 1 was due to the fact that they each had been starved of food and water, some considerable time before they were slaughtered. This may have caused a reduction in the extracellular fluid volume.

The mean value obtained for the water/fat-free body ratio of the pigs in experiment 3 agrees fairly closely with values obtained in previous studies (Kraybill et al., 1953; Clawson, Sheffy and Reid, 1955; Gnaedinger et al., 1963; Reid et al., 1968).

In the present study the coefficient of variation (C.V.) of the water/fat-free body ratio was calculated to be 1.26% which is similar to that (0.74%) obtained by Gnaedinger et al. (1963) but which is much smaller than the value (3.49%) obtained by Reid et al. (1968). The values obtained in the present work and in the study of Gnaedinger et al. (1963) were obtained from a small number of animals (24) of similar weight, age and breed. The value obtained by Reid et al. (1968) was calculated from the data of 714 pigs of different breed types, age and body size. It would seem, therefore, that in a more homogenous population of animals, with respect to breed, age and body size, there is only a small variation in the water content of the fat-free body and the use of a constant factor for predicting the weight of the fat-free body from body water determinations would not result in large errors of prediction. Conversely however, the use of a constant water/fat-free weight ratio in animals of widely differing ages and weights would almost certainly result in large errors of prediction. From the data obtained in experiment 3, the minimum error of estimate of fat-free weight from body water was calculated to be $\pm$ 0.730 kg. The regression equation is given:-

$$F.F.W\ (kg) = 1.33\ E.B.W - 0.350 \quad R.S.D = \pm 730\ kg \quad r = 0.992$$

The mean value of the fat-free weight was 59.38 kg and the C.V. about the mean is therefore 1.23%. These findings suggest that it would be of little value to improve on this accuracy of prediction of the fat-free weight from body water determinations in animals of similar type and weight.

It was also found that the potassium content of the fat-free body was extremely constant. In the pigs in experiment 1, the ratio was 0.268% (S.D. = ±0.0139%) and for the pigs in experiment 3 it was 0.269% (S.D. = ± 0.0063%). There were no significant differences ($P > 0.05$) between the sexes or between experiments and the pooled mean potassium/fat-free body ratio was calculated to be 0.269% (S.D. = ±0.0100%), the C.V being 3.72%. The value of this ratio agrees closely with those obtained in previous studies (Pfau, 1966; Stant, Martin and Kessler, 1969).

From the data obtained in experiment 3, it was found that the regression of the fat-free weight on empty body potassium had a R.S.D. of 1.31 kg, which was 2.21% of the mean fat-free weight. This indicates that it would be of little value to attempt to achieve greater accuracy of prediction of the fat-free weight from an estimation of body potassium than this, because of the natural variability in the potassium/fat-free body ratio. In populations of animals covering a wide range of weights and ages, these limits would possibly not be as close.

A prominent feature of the results obtained in experiments 1 and 3 was that the chemically-determined potassium was more closely associated with the fat-free dry weight than with the fat-free weight. A reverse situation was found for exchangeable potassium (K_e) determined by ^{42}K dilution. This was found to be more closely associated with the fat-free weight. This suggests that the proportion of body potassium which is readily exchangeable (i.e. in about 24 hours) with the labelled potassium, is more closely associated with those tissues having the greatest degree of hydration.

The mean crude protein content of the fat-free body was found to be 20.14% for the pigs in experiment 3. The S.D. was ±0.727% which was 3.61% of the mean value. This is a much lower C.V. than that

(12.2%) calculated from the data of Reid et al. (1968). Total body protein was found to be more closely associated with the body potassium ($r = 0.986$) than with the empty body water ($r = 0.904$) which indicates that body protein could be determined more accurately from an estimation of body potassium than body water. The regression equations relating body protein to empty body water and potassium are given:-

1. Protein (kg) = 0.269 E.B.W. - 0.125 R.S.D. = $\pm$ 0.561 $r = 0.904$
2. Protein (kg) = 0.080 K. - 0.877 R.S.D. = $\pm$ 0.220 $r = 0.986$

The C.V's of the regressions were 4.69% for equation 1 and 1.84% for equation 2, which indicates that the estimation of body potassium would give a more accurate prediction of body protein than would a determination of the body water.

Analysis of the data obtained in experiments 1 and 3 showed that there were no significant relationships between either the age, weight or fatness of the animal, and the composition of the fat-free body. Indeed, it was found that although there was a fairly large range in the weight of the fat-free material, this was not significantly correlated with the concentration of any of the constituents of the fat-free material. These results indicate that the pig has a remarkable capacity for maintaining constant the composition of the fat-free body when it is subjected to extreme nutritional regimes.

Although some previous work (Spray and Widdowson, 1951) has shown that age and body weight and even degree of fatness (Gnaedinger et al., 1963) are closely associated with the composition of the fat-free body, the number of animals involved in these analyses was not large. Indeed, from the literature there seems to be a lack of information regarding the extent to which the composition of the fat-free body, both within and

between breeds can be influenced by age, body weight and feeding regime. A comprehensive study is required in which a large number of animals of different breeds, reared on different nutritional regimes, are sacrificed and analysed at different stages of growth, to quantify any compositional changes which may occur in the fat-free body. It would possibly be more meaningful to analyse the results of each breed, age and weight group on a fat-free, moisture-free basis, rather than on a fat-free basis. The data obtained for the pigs in experiments 1 and 3 indicate that the variability of the fat-free constituents of the body was smaller when expressed on a fat-free, moisture-free basis, than on a fat-free basis.

(ii) In the developmental part of this investigation several indirect methods for estimating in vivo body composition were investigated. These were dilution techniques, potassium 42 (^{42}K), Deuterium oxide (D_2O), Evans Blue, urea and sodium thiocyanate. Of these, only the ^{42}K, D_2O and Evans Blue dilution techniques were considered suitable for inclusion into the second part of the investigation, in which they were applied in combination with other indirect techniques.

Potassium 42 was found to give extremely accurate estimates of the major chemical components of the body. Despite the fact that the tracer was very expensive (approximately £9/mC) and sample preparation was fairly laborious, the method was investigated further because it was considered that it could provide relatively useful information on the composition of the body in those circumstances in which the facilities for its handling and analysis were available, and in which high accuracy was required.

The results obtained for the D_2O technique were generally disappointing, but there was some evidence to suggest that under more controlled conditions of sample preparation and analysis, the method could predict body water fairly closely. Also, the D_2O technique has

the advantage that it is non-radioactive, but the tracer is fairly expensive, laborious to analyse, and requires expensive analytical equipment. For this reason a cheaper body water tracer, urea, was investigated, but this gave some very unreliable results and was not considered suitable for inclusion in the second part of the study.

The Evans Blue technique gave some fairly repeatable estimates of plasma and blood volume in one sow in which it was investigated. The method was simple and the tracer was very cheap, and it was considered that if it gave estimates of blood volume which were closely associated with the fat-free weight, then it could be used in those situations in which a large number of animals were to be scrutinised and in which there were limited technical resources.

In this part of the study also, an attempt was made to estimate extracellular fluid volume by sodium thiocyanate dilution. It was considered that in combination with an estimate of the total body water, an extremely accurate estimate of the fat-free weight could be made, as previous studies had already indicated (McCance and Widdowson, 1951; Hörnicke, 1961). The thiocyanate dilution technique was chosen because the method of analysis is relatively simple and the tracer is not expensive. The results which were obtained, however, were not considered reliable when compared with D_2O estimates of total body water, and therefore investigation of the technique was discontinued. Possibly, the application of other tracer substances such as Bromine 82, thiosulphate or inulin could give reliable estimates of extracellular volume in pigs.

The objective of the second part of the investigation was to provide data so that the estimates of body composition, provided by several indirect methods, when applied singly or in combination to animals of the same liveweight, could be compared. The indirect techniques used were

^{42}K, D_2O and Evans Blue dilution, ultrasonics, measurements of external body dimensions, visual appraisal and the corrected food conversion ratio (C.F.C.R.). These methods were intended to cover a wide range of sophistication and expense so that individually they could be used in a wide range of practical situations.

From the results which were obtained for each of these methods, it is possible to class them into any one of three groups depending on the proportion of body component measured.

In the first group, ultrasonics, external measurements and visual appraisal are included. These are techniques which measure only a relatively small part of a body component, and the assumption is that this bears a constant relationship with the rest of the component. From the results which were obtained in the second part of the investigation it is clear that large errors can result in the estimation of body components when measurements are made on only a small proportion of the components. For example, ultrasonic measurements of backfat in various positions gave relatively poor estimates of total body lipid and dissectible fat, although the accuracy of prediction of the latter was greater than for body lipid. Apart from the technical errors of measurement it was considered that the variable amount of internal fat to be the largest source of error in the prediction of body lipid by ultrasonics.

In the second group are the dilution techniques which measure the size of whole body components. Thus ^{42}K estimated body potassium, D_2O estimated body water, and Evans Blue gave an estimate of blood volume.

A major assumption in the application of such techniques is that the tracer becomes entirely dispersed in the whole of the body component. From the results which were obtained in experiments 1 and 3, it was found that ^{42}K had equilibrated with only about 97% of the total body potassium

in about 24 hours, and that D_2O not only equilibrated with the water in the empty body, but also equilibrated with the water in the gastro-intestinal tract and possibly with some of the non-aqueous labile hydrogen ions in protein and fat. For the Evans Blue dilution technique it is impossible to assess its accuracy of prediction of blood volume, as no direct estimates were made.

Of these three methods which were applied in experiment 3, ^{42}K and D_2O dilution gave the most accurate estimates of the major chemical and dissectible components of the body. The accuracy of prediction of the dissectible components was not as good as for the chemical components, presumably because the dissectible components did not form constant fractions of the total component in the body, and also contained variable amounts of other components.

In contrast to these methods the Evans Blue dilution technique was relatively simple and inexpensive, but it gave relatively poor estimates of the body components. From the results obtained in experiment 3 it was found that the estimated blood volume was relatively poorly associated with the fat-free weight ($r = 0.819$) and dissectible lean ($r = 0.830$). Neither of these two body components can be considered to be strictly representative of the 'active protoplasmic mass'. The dissectible lean did not represent all of the lean tissue mass of the body, and the fat-free body included of course, a significant proportion of 'non-active' material — mineral. The ash-free, fat-free mass was considered to be the most indicative of the 'active mass', but its relationship with the estimated blood volume was also relatively poor, the correlation being only 0.830.

In the third group of methods which included carcass specific gravity and the measurement of C.F.C.R., the relative proportions of the two major components of the body, fat-free body and lipid, were measured.

One of the major sources of error in the determination of *in vivo* body composition from the estimation of only *one* body component arises from the assumption that there is a strictly constant relationship between that component and either the fat-free weight or body lipid. The application of indirect methods which provide an indication of the relative proportions of the two major components - lipid and fat-free material may reduce this error to some extent.

The C.F.C.R. measurements gave remarkably accurate estimates of the composition of the pigs in experiment 3, despite the number of assumptions which were made in making the adjustments to the feed conversion ratio figure. Before applying the method further, the effects of factors such as sex, breed and type of feeding system, on the predictive accuracy of the technique require investigation. A further study is also required to compare the relative accuracy of prediction equations involving C.F.C.R. calculated over a weight range 25-90 kg, with those in which C.F.C.R. is estimated over smaller weight ranges.

Nevertheless, it would seem from the results obtained in this investigation that the measurement of C.F.C.R. could easily be made in situations where individual feeding accommodation already exists and could provide a basis on which pigs on a performance test could be ranked according to their conversion of feed to lean tissue.

The results obtained for the specific gravity technique showed, as have several previous studies, that the measurement of the density of the carcass can provide an extremely accurate estimate of carcass composition. The factors which influence carcass density are the relative proportions of fat-free tissue and lipid, and to some extent the degree of mineralisation of the skeleton. Calculations showed, however, that the dissectible lean/dissectible fat ratio was highly correlated with specific gravity but that the dissectible lean/dissectible bone ratio

was not significantly ($P > 0.05$) correlated with carcass specific gravity.

A possible source of error in the method as it was applied in this investigation lies in the assumption that the specific gravity of one side of the carcass bears a constant relationship with specific gravity of the whole carcass. By measuring the specific gravity of only one side there is a possibility that the unequal splitting of the carcass down the centre of the vertebral column could have resulted in an uneven distribution of bone between the two sides. Apart from this there is also the possibility of assymetry in the deposition of fat between the two sides.

From the results obtained in this investigation it seems that, despite these possible sources of error, the measurement of carcass specific gravity can provide an extremely accurate estimate of body composition and, extending this to live animals, the development of a reliable technique for measuring body volume could possibly provide very accurate estimates of body composition in combination with body weight.

In general terms it has been shown from the data obtained in this investigation, that a marked improvement in the accuracy of prediction of *in vivo* body composition can be expected as a greater proportion of a body component is measured. At one extreme there are those techniques which measure only a relatively small part of a component and give relatively poor estimates of body composition. At the other extreme there are those methods which give an indication of the size of two components and provide relatively accurate estimates of composition.

The logical extension of this theme is the simultaneous measurement of several components of the body by the application of different indirect methods. Indeed, from the results obtained in experiment 3, in which several techniques were simultaneously applied to each animal, it was

found that exceptionally accurate estimates of body composition were obtained by combinations of predictors which measured different components of the body. The relative predictive efficiencies of several predictors used singly and in combination for predicting the fat-free weight are given in the following table. The R.S.D. of the estimate of the fat-free weight from ^{42}K dilution is given as 100%.

The relative predictive efficiencies of several predictors used singly and in combination, for predicting fat-free weight (R.S.D. of ^{42}K estimate = 100%)

Predictor	Relative Predictive Efficiency (%)
(a) Single	
^{42}K	100
D_2O	71.8
C.F.C.R.	67.1
Evans Blue	39.8
Forearm length	32.5
(b) Combinations	
^{42}K, C.F.C.R.	151.7
^{42}K, S.F.US	124.7
^{42}K, D_2O	113.0
C.F.C.R., Jaw length	73.8
Neck circumference, L.F.US	56.7

It can be seen from this table that most of the combinations of predictors gave more accurate estimates of the fat-free weight than did the most accurate single predictor - ^{42}K, in isolation. Also, relatively simple and expensive measurements such as C.F.C.R. and the measurement of shoulder fat depth by ultrasonics, in combination with ^{42}K, improved the accuracy of prediction of the fat-free weight from about 25 to 52% over the use of ^{42}K alone.

It would also seem that more accuracy in the prediction of one component can be achieved by using combinations of predictors each of which measures different components of the body (e.g. fat-free body by ^{42}K dilution and body lipid by ultrasonics) rather than the simultaneous measurement of the same component, (e.g. fat-free body by ^{42}K and D_2O dilution).

In conclusion, therefore, the results of this investigation have shown that the accuracy of prediction of body composition depends on the type of method used and on the number of methods which are applied simultaneously.

Some of the more sophisticated techniques provide extremely accurate estimates of body composition, but for practical application this must be balanced against the expense which the method incurs and also against the accuracy which is required in the investigation. Indeed, as was found after re-synthesis of the data, it is possible that in some situations a combination of two or more simple predictors could give the same degree of accuracy as the application of one sophisticated technique without involving as much expense.

It seems that when two or more predictors are used in combination, the accuracy of the estimate depends on the number of body components which are measured. It was found for example that two different estimates of the same component did not give as much information on that component as when one of the predictors used, measured another component.

(b) <u>The relevance of the findings of the present investigation to practical situations</u>

The accuracy of prediction of body composition in practical situations is often limited by the facilities, equipment and expertise which are available, and very often little regard is given to the cost-effectiveness of the method which is applied. Thus in many circumstances the accuracy of prediction of body composition required is not matched by that of the indirect method which is applied and the method is

considered either too expensive or too inaccurate.

That it would seem that for the application of an indirect method to practical situations, attention must be given to (i) the suitability of the method in the situation (ii) the body component which is being measured.

(i) Each of the indirect methods which were investigated in experiment 3 is not suitable for application in many practical situations because either it cannot identify real differences in composition, or it involves a complex analytical procedure which is too expensive for routine use. The simple and inexpensive techniques such as ultrasonics have been shown to give relatively poor estimates of body composition, because they measure only small proportions of body components. These methods, however, are possibly of value for ranking animals on the basis of carcass quality, and of course a large number of animals can be scrutinised at little expense.

With such a versatile technique as ultrasonics, further research could be directed towards improving its accuracy by integrating a series of backfat measurements made in several planes radially to the longitudinal axis of the pig. The AN/SCAN machine developed by Stouffer attempts this, which is an extension of the thesis that an improvement in accuracy can be obtained by measuring a larger proportion of a body component.

Generally, dilution techniques have been shown to give relatively accurate estimates of body composition. Much of the accuracy of dilution techniques depends on the precision with which the tracer is injected, and the accuracy of measurement of the tracer in an easily accessible body fluid. In routine applications, therefore, special precautions may have to be observed to ensure that these conditions are fulfilled.

The use of radio-active tracers in dilution studies incurs additional expense. Special facilities and equipment for the handling

and analysis of radio-isotopes are required and in order to minimise pollution of the environment, isotopes of high purity and short half-lives are preferred.

The radio-isotope, Potassium 42, is one example of a tracer with a short half-life. It has been shown to give extremely accurate estimates of body composition. In practical terms, its use would possibly be restricted because of its high cost and the requirement for special facilities and equipment for its handling and analysis. Also its use would almost certainly be restricted at large distances away from the source of manufacture, because of the short half-life. However, the method offers several advantages over the potassium 40 counting technique in that the technique can be taken to the animal rather than vice versa, the requirement for skilled personnel is low, and several animals can be analysed simultaneously. The applicability of the ^{42}K technique could possibly be improved by the oral administration of the tracer and the analysis of body fluids for γ-radiation.

Deuterium oxide has the advantage over ^{42}K in that it is a non-radioactive tracer. This means that it can be used in most types of housing at any distance from the source of manufacture, and it can be stored quite easily provided that precautions are taken to prevent its contamination by water. It has been shown to be a fairly accurate technique in pigs, but possibly the greatest source of error is the variable amount of water in the gastro-intestinal tract. Obviously for the application of the method to ruminants, the effect of intestinal fill would assume much greater importance than it does in pigs. Deuterium oxide is relatively expensive (approximately £55/kg) and requires an elaborate procedure for its analysis in body fluids. It may be possible to reduce the analytical cost per animal by taking only one sample of body fluid after equilibrium, but the effect of this on the accuracy

of the technique would need investigation.

The measurement of corrected feed conversion was found to give estimates of body composition of comparable accuracy to the D_2O dilution technique. Two major advantages of the method are its simplicity and low cost, and therefore it could be used in a wide range of situations, providing that individual feeding accommodation was available. However, it would only be of use for estimating body composition at the end of periods of growth and would not be suitable for measuring sequential changes in composition. It would therefore seem to be an ideal method for use in performance testing schemes, and even greater accuracy could be obtained by combining with it another relatively simple measurement, ultrasonic backfat depth.

(ii) In many situations in which a determination of *in vivo* body composition is required, little attention is given to the body component which is being measured, and its relationship with the remainder of the body.

From a review of the literature it seems that the fat-free body is a concept which is frequently used in many studies on body composition. The methods frequently used to determine the weight of the fat-free body are those in which body water, or body potassium are determined, and from these estimates the proportions of other constituents such as lipid and nitrogen can be determined. However, considerable errors can result in the determination of the weight of body lipid from the fat-free weight, because of the effect of gut-fill. These errors could be reduced by either simultaneously applying two tracers, from which the size of the gut contents could be determined or by estimating body lipid directly by inert-gas dilution. Unfortunately, there has been relatively little research towards developing techniques which achieve either of these objectives.

A problem of similar magnitude is that the size of the fat-free body gives little indication of the relative proportions of the different commercial joints in the carcass. It can be postulated that the determination of body lipid by inert-gas dilution would also give little indication of the distribution of fat in the carcass.

It would seem, therefore, that there is an obvious requirement for the development of methods which will give information on the size of certain internal dimensions of the body. Existing methods such as X-ray photography and ultrasonics could be further developed for this purpose, and which in combination with other techniques could give a reliable assessment of the live animal in commercial terms.

A criticism of body composition research at the present time is that much of the effort is given to the improvement of precision of existing methods which can only be used in certain situations, and relatively little attention is given to the development of new methods, and to the modification of existing ones.

From the results obtained in this investigation it seems that combinations of certain predictors can give extremely accurate estimates of body composition in a wide range of situations, but more research is required for the development of new predictors from which the composition of the body can be assessed, both in fundamental and in commercial terms.

ADAM, J.L. and SMITH, W.C. (1964).
Anim. Prod. 6, 97.

ALSMEYER, R.H., HINER, R.L. and THORNTON, J.W. (1963).
Ann. N.Y. Acad. Sci. 110, 23.

ANDERSON, D.M. (1967).
Ph.D. Thesis, University of Aberdeen.

ANDERSON, D.M. and ELSLEY, F.W.H. (1969).
J. Agric. Sci., Camb. 72, 475.

ANDERSON, D.M., McDONALD, I. and ELSLEY, F.W.H. (1969).
J. Agric. Sci., Camb. 73, 501.

ANDERSON, E.C. (1959).
Fd. Res. 24, 605.

ANDERSON, E.C. (1963).
Ann. N.Y. Acad. Sci. 110, 189.

ANDREWS, F.N. and WHALEY, R.M. (1955).
Rep. Ind. Agric. Exp. Sta. No. 68: 27.

A.O.A.C. (1965).
Official Methods of Analysis of the Official Agricultural Chemists.

ASHWORTH, U.S. and COWGILL, G.R. (1938).
J. Nutr. 15, 73.

BAILEY, C.B., KITTS, W.D. and WOOD, A.J. (1960).
Canad. J. Anim. Sci. 40, 143.

BANFIELD, F.H. and CALLOW, E.H. (1935).
J. Soc. Chem. Ind. (Lond.) 54, 413T.

BEHNKE, A.R. (1943).
Harvey Lectures (1941-42), 37, 198.

BEHNKE, A.R., THOMSON, R.M. and SHAW, L.A. (1935).
Am. J. Physiol. 114, 137.

BERG, R.T. and BOWLAND, J.P. (1956).
Res. Bull. Alberta Univ. Ext. Dept. 41 (2) 21.

BERNARD, C. (1878).
"Leçons sur les phenomenes de la vie", 2, 7. Paris.

BLAXTER, K.L. (1955).
A.R.C. Sub-Committee on Pig Nutrition. 491/55.

BLAXTER, K.L. and ROOK, J.A.F. (1956).
J. Agric. Sci., Camb. 48, 194.

BLUMBERG, A.G., LA DU, B.M., LESSER, G.T. and STEELE, J.M. (1952).
J. Pharm. Exptl. Ther. 104, 325.

BOLING, E.A. and LIPKIND, J.B. (1963).
J. Appl. Physiol. 18, 943.

BOLING, E.A., TAYLOR, W.L., ENTENMAN, C. and BEHNKE, A.R. (Jr.) (1962).
J. Clin. Invest. 41, 1840.

BOURDILLON, J. and LAVIETES, P.H. (1936).
J. Clin. Invest. 15, 301.

BOWLER, R.G. (1944).
Biochem. J. 38, 385.

BRADBURY, M.W.B. (1961).
Brit. J. Nutr. 15, 177.

BRATZLER, L.J. and MARGERUM, E.P. (1953).
J. Anim. Sci. 12, 856.

BREIREM, K. (1939).
Tierernährung 11, 487.

BRINKMAN, G.L. (1965).
Pediatrics 36, 94.

BRODIE, B.B. (1951).
Methods in Med. Res. 4, 31.

BRODIE, B.B., BERGER, E.Y., AXELROD, J., DUNNING, M.F., POROSOWSKA, Y. and STEELE, J.M. (1951).
Proc. Soc. Exptl. Biol. Med. 77, 794.

BRODIE, B.B., BRAND, E. and LESHIN, S. (1939).
J. Biol. Chem. 139, 555.

BROZEK, J. and HENSCHEL, A. (Eds.) (1961).
Techniques for Measuring Body Composition.
Washington, D.C., National Academy of Sciences.

BRÜGGEMANN, H. (1940).
seen in Anim. Breeding Absts. 10, 172.

BUCK, S.F., HARRINGTON, G. and JOHNSON, R.F. (1962).
Anim. Prod. 4, 25.

BURCH, P.R.J. and SPIERS, F.W. (1953).
Nature, London 172, 519.

BUSH, J.A., JENSEN, W.W., CARTWRIGHT, G.E. and WINTROBE, M.M. (1955).
Am. J. Physiol. 181, 9.

CALLOW, E.H. (1947).
J. Agric. Sci., Camb. 37, 113.

CANNON, W.B. (1929).
Physiol. Rev. 9, 399.

CARDOZO, R.H. and EDELMAN, I.S. (1952).
J. Clin. Invest. 31, 280.

CIZEK, L.J. (1954).
Am. J. Physiol. 179, 104.

CLAWSON, A.J., SHEFFY, B.E. and REID, J.T. (1955).
J. Anim. Sci. 14, 1122.

COHN, K. (1947).
in "Preparation and Measurement of Isotopic Tracers"- A symposium prepared for the Isotope Research Group. Ann Arbor, Michigan, p. 57.

COOK, G.L. and CUTHBERTSON, A. (1967).
Anim. Prod. 9, 278.

CORSA, L. OLNEY, J.M., STEENBURG, R.W., BALL, M.R. and MOORE, F.D. (1950).
J. Clin. Invest. 29, 1280.

COURTICE, F.C. (1943).
J. Physiol. Lond. 102, 290.

CRANDALL, L.A. and ANDERSON, M.X. (1934).
Am. J. Digest. Diseases 1, 126.

DAVIDSSON, D., MACINTYRE, I., RAPOPORT, A. and BRADLEY, J.E.S. (1956).
Biochem. J. 62, 34P.

DAVIDSON, J., MATHIESON, J. and BOYNE, A.W. (1970).
Analyst, 95, 181.

DE PAPE, J.G. and WHATLEY, J.A. (1956).
J. Anim. Sci. 15, 1029.

DIGGS, B.G., BECKER, D.E., TERRILL, S.W. and JENSEN, A.H. (1959).
J. Anim. Sci. 18, 1492.

DOORNENBAL, H. (1962).
Dissertation Absts. 22, 3244.

DOORNENBAL, H. and MARTIN, A.H. (1965).
Canad. J. Anim. Sci. 45, 203.

DOORNENBAL, H., ASDELL, S.A. and WELLINGTON, G.H. (1962).
J. Anim. Sci. 21, 461.

DOXIADIS, S.A. and GAIRDNER, D. (1948).
Clin. Sci. 6, 259.

D'SILVA, J.L. and NEIL, M.W. (1951).
Biochem. J. 49, 222.

DUFFUS, G.M. (1969).
Ph.D. Thesis, University of Aberdeen.

DUMONT, B.L. (1955).
Annls. Inst. Natn. Rech. agron. Paris. Sér. D. Annls. Zootech. 4, 285

EAST, T., TAYLOR, J., MILLER, I.T. and WIDDOWSON, R.W. (1959).
Anim. Prod. 1, 129.

EDELMAN, I.S. (1961).
in "Techniques for Measuring Body Composition." Ed. by J. Brožek and A. Henschel. Washington, D.C. National Academy of Sciences.

EDELMAN, I.S., OLNEY, J.M., JAMES, A.H., BROOKS, L. and MOORE, F.D. (1952).
Science 115, 447.

ELRICK, R.H. and PARKER, R.P. (1968).
Int. J. Appl. Radiation Isot. 19, 263.

ELSLEY, F.W.H., MCDONALD, I. and FOWLER, V.R. (1964).
Anim. Prod. 6, 141.

FALLER, I.L., BOND, E.E., PETTE, D. and PASCALE, L.R. (1955).
J. Lab. Clin. Med. 45, 759.

FENGER-ERIKSON, K.A., KROGH, A. and USSING, H. (1936).
Biochem. J. 30, 1264.

FENN, W.O., NOONAN, T.R., MULLINS, L.J. and HAEGE, L. (1941).
Am. J. Physiol. 135, 149.

FINE, J. and SELIGMAN, A.M. (1943).
J. Clin. Invest. 22, 285.

FLEAR, C.T.G., COOKE, W.T., SIVYER, A. and DOMONET, J. (1963).
Clinica chim. Acta, 8, 768.

FLEXNER, L.B., GELLHORN, A. and MERRELL, M. (1942).
J. Biol. Chem. 144, 35.

FLYNN, M.A., HANNA, F., LONG, C.H., ASFOUR, R.Y., LUTZ, R.N. and ZOBRISKY, S.E. (1968).
in "Body Composition in Animals and Man". Publ. No. 1598. Washington, D.C. National Academy of Sciences.

FOMAN, S.J., JENSEN, R.L., OWEN, G.M. (1963).
Ann. N.Y. Acad. Sci. 110, 80.

FOOT, J.Z. (1969).
Ph.D. Thesis, University of Aberdeen.

FORBES, G.B. (1955).
Growth, 19, 75.

FORBES, G.B., GALLUP, J. and HURSH, J.B. (1961).
Science, N.Y. 133, 101.

FORBES, R.M. (1962).
Pediatrics 29, 477.

FORBES, R.M., COOPER, A.R. and MITCHELL, H.H. (1953).
J. Biol. Chem. 203, 359.

FOWLER, V.R. (1966).
in Proc. 9th Internat. Congr. Anim. Prod. Edinburgh.
Oliver and Boyd Ltd.

FOWLER, V.R. (1967).
in "Growth and Development of Mammals." Proc. 14th Nottingham Easter School. London, Butterworths.

FRIES, G.F. and LYNCH, G.P. (1968).
in "Body Composition in Animals and Man." Publ. No. 1598.
Washington, D.C. National Academy of Sciences.

GAMBLE, J.L. Jnr., ROBERTSON, J.S., HANNIGAN, C.A., FOSTER, C.G. and FARR, L.E. (1953).
J. Clin. Invest. 32, 483.

GARRETT, W.N., MEYER, J.H. and LOFGREEN, G.P. (1959).
J. Anim. Sci. 18, 116.

GIBSON, J.G., SELIGMAN, A.M., PEACOCK, W.C., AUB, J.C., FINE, J. and EVANS, R.D. (1946).
J. Clin. Invest. 25, 848.

GILMAN, A., PHILIPS, F.S. and KOELLE, E.S. (1946).
Am. J. Physiol. 146, 348.

GINSBURG, J. and WILDE, W.S. (1952).
Federation Proc. 11, 54.

GNAEDINGER, R.H., PEARSON, A.M., REINEKE, E.P. and HIX, V.M. (1963).
J. Anim. Sci. 22, 495.

GNAEDINGER, R.H., REINEKE, E.P., PEARSON, A.M., VAN HUSS, W.D., WESSEL, J.A. and MONTOYE, H.J. (1963).
Ann. N.Y. Acad. Sci. 110, 96.

GOLDMAN, D.E. and HEUTER, T.F. (1956).
J. acoust. Soc. Am. 28, 35.

GOPALAN, C., SRIKANTIA, S.G. and VENKATACHALAM, P.S. (1955).
Metabolism, 2, 335.

GREGERSON, M.I. and RAWSON, R.A. (1943).
Am. J. Physiol. 138, 698.

GREGERSON, M.J. and RAWSON, R. (1959).
Physiol. Rev. 39, 307.

GROVE, E.L., JONES, R.A. and MATTHEWS, W. (1961).
Anal. Biochem. 2, 221.

GROVES, T.D.D. and WOOD, A.S. (1968).
Canad. J. Anim. Sci. 45, 14.

HABERER, K. (1965).
Atom. Wirtsch. 10, 36.

HAIGH, C.P. (1953).
Nature, London 172, 359.

HAIGH, C.P. and SCHNEIDER, H. (1956).
J. Physiol. Lond. 131, 377.

HAMMOND, J. (1932).
Growth and Development of Mutton Qualities in the Sheep.
Edinburgh, Oliver and Boyd.

HANKINS, O.G. and ELLIS, N.R. (1934).
J. Agric. Res. 48, 257.

HANKINS, O.G. and HOWE, P.E. (1946).
Tech. Bull. U.S. Dept. Agric. No. 926.

HANSARD, S.L., SAUBERLICH, H.E. and COMAR, C.L. (1951).
Proc. Soc. Exptl. Biol. Med. 78, 544.

HARRINGTON, G. (1958).
"Pig Carcass Evaluation". Comm. Bur. Anim. Breeding & Genetics, T.C.12
C.A.B., Farnham Royal, Bucks. England.

HARRINGTON, G. (1963).
Ann. N.Y. Acad. Sci. 110, 642.

HARRIS, E.J. (1952).
J. Physiol. Lond. 117, 278.

HARRISON, H.E., DARROW, D.C. and YANNET, H. (1936).
J. Biol. Chem. 113, 515.

HATAI, S. (1917).
Amer. J. Anat. 21, 23.

HAZEL, L.N. and KLINE, E.A. (1952). J. Anim. Sci. 11, 313.

HAZEL, L.N. and KLINE, E.A. (1959).
J. Anim. Sci. 18, 815.

HEDRICK, H.B., MEYER, W.E., ALEXANDER, M.A., ZOBRISKY, S.E. and NAUMANN, H.D. (1962).
J. Anim. Sci. 21, 362.

HEVESY, G. and HOFER, E. (1934).
Nature, London 134, 879.

HEVESY, G. and JACOBSEN, C.F. (1940).
Acta physiol. scand. 1, 11.

HILL, F. and O'Carroll, F.M. (1962).
Irish J. Agric. Res. 1, 115.

HIX, V.M., PEARSON, A.M., REINEKE, E.P. (1964).
J. Appl. Physiol. 19, 955.

HOGREVE, F. (1942).
Z. Tierz. ZüchtBiol. 54, 1.

HOLLANDER, V., CHANG, P. and CO TUI (1949).
J. Lab. Clin. Med. 34, 680.

HOLME, D.W., COEY, W.G., ROBINSON, K.L. (1963).
J. Agric. Sci. Camb. 61, 9.

HÜRNICKE, H. (1959). Methoden zur Bestimmung der Korperzusammensetzung Lebender Schweine

HÜRNICKE, H. (1961).
Z. Tierphysiol. Tierernähr. Futtermittelk. 16, 344.

HUCKABEE, W.E. (1956).
J. Appl. Physiol. 9, 157.

HURST, W.W., SCHEMM, F.R. and VOGEL, W.C. (1952).
J. Lab. Clin. Med. 39, 36.

HUXLEY, J.S. (1932).
Problems of Relative Growth. London, Methuen.

HYDÉN, E.S. (1956). K. LantbrHögsk. Annlr. 22, 139

HYTTEN, F.E. (1964).
Proc. Nutr. Soc. 23, xxi.

HYTTEN, F.E. (1969).
Personal communication.

HYTTEN, F.E. and PAINTIN, D.B. (1963).
J. Obstet. Gynaecol. Br. Commonwealth, 70, 402.

HYTTEN, F.E., TAGGART, N., BILLEWICZ, W.Z. and JASON, J.C. (1962).
Physics Med. Biol. 6, 415.

HYTTEN, F.E., TAYLOR, K. and TAGGART, N. (1966).
Clin. Sci. 31, 111.

JOBLIN, A.D.H. (1966).
N.Z. J. Agric. Res. 9, 227.

JOHNSON, J.E., HARTSUCK, J.M., ZOLLINGER, R.M. Jnr. and MOORE, F.D. (1969).
Metabolism 18, 663.

JUDGE, M.D., STOB, M., KESSLER, W.V. and CHRISTIAN, J.E. (1963).
J. Anim. Sci. 22, 418.

KALTREIDER, N.L., MENEELY, G.R., ALLEN, J.R. and BALE, W.F. (1941).
J. Exptl. Med. 74, 569.

KAY, M. (1963).
Ph.D. Thesis, University of Aberdeen.

KAY, M., JONES, A.S. and SMART, R. (1966).
Br. J. Nutr. 20, 439.

KAY, M., MACDEARMID, A. and MACLEOD, N.A. (1970).
Animal Prod. 12, 261.

KESTON, A.S., RITTENBERG, D. and SCHOENHEIMER, R. (1937-38).
J. Biol. Chem. 122, 227.

KEYS, A. and BROŽEK, J. (1953).
Physiol. Revs. 33, 245.

KIELANOWSKI, J. (1965).
Proc. Symp. Energy Metabolism, Troon, Scotland, 1964.
(Publ. European Ass. Anim. Prod. No. 11).

KIELANOWSKI, J. (1966).
Anim. Prod. 8, 121.

KIRTON, A.H. and BARTON, R.A. (1958).
J. Agric. Sci. Camb. 51, 265.

KIRTON, A.H., GNAEDINGER, R.H. and PEARSON, A.M. (1963).
J. Anim. Sci. 22, 904.

KIRTON, A.H. and PEARSON, A.M. (1963a).
J. Anim. Sci. 22, 125.

KIRTON, A.H. and PEARSON, A.M. (1963b).
Ann. N.Y. Acad. Sci. 110, 221.

KIRTON, A.H., PEARSON, A.M., NELSON, R.H., ANDERSON, E.C. and SCHUCH, R.L. (1961).
J. Anim. Sci. 20, 635.

KIRTON, A.H., PEARSON, A.M., PORTER, R.W. and NELSON, R.H. (1961).
J. Fd. Sci. 26, 475.

KLIESCH, J.U., NEWHAUS, E., SILBER, E. and KOSTZEWSKE, E. (1957).
Ztschr. Tierz. Zuchtungsbiol. 70, 29.

KODOMA, A.M. and PACE, N. (1963).
J. Appl. Physiol. 18, 1272.

KRAINTZ, L., DE BOER, J., SMITH, E.L. and HUGGINS, R.A. (1958).
Am. J. Physiol. 195, 628.

KRAYBILL, H.F., BITTER, H.L. and HANKINS, O.G. (1952).
J. Appl. Physiol. 4, 575.

KRAYBILL, H.F., GOODE, E.R., ROBERTSON, R.S.B. and SLOANE, H.S. (1953).
J. Appl. Physiol. 6, 27.

KROGH, A. and USSING, H.H. (1936).
Skand. Arch. f. Physiol. 75, 90.

KULWICH, R., FEINSTEIN, L. and ANDERSON, E.C. (1958).
Science, N.Y. 127, 338.

KULWICH, R., FEINSTEIN, L. and GOLUMBIC, C. (1960).
J. Anim. Sci. 19, 119.

KULWICH, R., FEINSTEIN, L., GOLUMBIC, C., HINER, R.L., SEYMOUR, W.R. and KAUFFMAN, W.R. (1961).
J. Anim. Sci. 20, 497.

KUMAR, I. (1957).
Ph.D. Thesis, University of Aberdeen.

LADEFOGED, J. and ANDERSON, A.M. (1967).
Phys. Med. Biol. 12, 353.

LAUPRECHT, E., SCHEPER, J. and SCHROEDER, J. (1957).
Mitt. der Deutsch. Landwirtschaftsgesellschaft , 72, 881.

LAVIETES, P.H., BOURDILLON, J., PETERS, J.P. and KLINGHOFFER, K.A. (1936).
J. Clin. Invest. 15, 261.

LAWES, J.B. and GILBERT, J.H. (1859).
Phil. Trans. Pt. II, 149, 494.

LAWRENCE, J.H., LOOMIS, W.F., TOBIAS, C.A. and TURPIN, F.H. (1946).
J. Physiol. Lond. 105, 197.

LAWRIE, R.A. (1966).
"Meat Science". 1st ed. London, Pergamon Press Ltd.

LESSER, G.T., BLUMBERG, A.G. and STEELE, J.M. (1952).
Am. J. Physiol. 169, 545.

LESSER, G.T. and DEUTSCH, S. (1967).
J. Appl. Physiol. 23, 621.

LESSER, G.T., PERL, W. and STEELE, J.M. (1960).
J. Clin. Invest. 39, 1791.

LESSER, G.T. and ZAK, G. (1963).
Ann. N.Y. Acad. Sci. 110, 40.

LEVITT, M.F. and GAUDINO, M. (1949).
Am. J. Physiol. 159, 67.

LEVITT, M.F. and GUADINO, M. (1950).
Am. J. Med. 9, 208.

LIGHT, A.E., SMITH, P.K., SMITH, A.H. and ANDERSON, W.E. (1934).
J. Biol. Chem. 107, 689.

LJUNGGREN, H. (1955).
Acta physiol. scand. 33, 69.

LJUNGGREN, H. (1959).
Acta Endocrinol. Suppl. No. 30.

LUCAS, I.A.M. and CALDER, A.F.C. (1956).
J. Agric. Sci. Camb. 53, 136.

LUCAS, I.A.M., MCDONALD, I. and CALDER, A.F.C. (1960).
J. Agric. Sci. Camb. 54, 81.

LUSH, J.L. (1926).
J. Agric. Res. 32, 727.

MANERY, J.F. (1954).
Physiol. Rev. 34, 334.

MARSH, W.H., FINGERHUT, B. and MILLER, H. (1965).
Clin. Chem. 11, 624.

MCCANCE, R.A. and WIDDOWSON, E.M. (1951).
Proc. Roy. Soc. B 138, 115.

MCFADDEN, P.L. and RICHARDS, C.R. (1956).
J. Dairy Sci. 39, 1438.

MCKENZIE, A. and TINDLE, J. (1959).
Lancet, 1, 333

MCMEEKAN, C.P. (1940).
J. Agric. Sci. Camb. 30, 276.

MCMEEKAN, C.P. (1941).
J. Agric. Sci. Camb. 31, 1.

MILLER, A.T. and BLYTH, C.S. (1952).
J. Appl. Physiol. 5, 73.

MILLER, C.E. and MARINELLI, L.D. (1956). Science, 124, 122.

MILLER, C.E. and REMENCHIK, A.P. (1963).
Ann. N.Y. Acad. Sci. 110, 175.

MITCHELL, H.H., HAMILTON, T.S., STEGGARDA, F.R. and BEAN, W.H. (1945).
J. Biol. Chem. 158, 625.

MONTGOMERY, P.O'B. and FOGELMAN, M.J. (1950).
Federation Proc. 9, 91.

MOORE, F.D. (1946).
Science, 104, 157.

MOORE, F.D., OLESEN, K.H., MCMURRAY, J.D., PARKER, H.V., BALL, M.R. and BOYDEN, C.M. (1963).
"The Body Cell Mass and its Supporting Environment."
Philadelphia, Pa., Saunders.

MORALES, M.F., RATHBUN, E.N., SMITH, R.E. and PACE, N. (1945).
J. Biol. Chem. 158, 677.

MORRIS, J. and MOIR, K.W. (1964).
in "Carcass Composition and Appraisal of Meat Animals" (Ed. D.E. Tribe).
Melbourne, The Commonwealth Scientific and Industrial Research Organization.

MOULTON, C.R. (1923).
J. Biol. Chem. 57, 79.

MULDOWNEY, F.P., CROOKS, J. and BLUHM, N.M. (1957).
J. Clin. Invest. 36, 1375.

MURRAY, J.A. (1922).
J. Agric. Sci. Camb. 12, 103.

NICKERSON, J.L., SEAR, H. and REEVE, E.B. (1953).
Am. J. Physiol. 175, 230.

NICOL, A.M., THOMSON, G.G. and MCLEAN, J.W. (1968).
N.Z. J. Agric. Res. 11, 330.

NOMOF, N., HOPPER, J.J., BROWN, E., SCOTT, K. and WENNESLAND, R. (1954).
J. Clin. Invest. 33, 1382.

NOONAN, T.R., FENN, W.O. and HAEGE, L. (1941).
Am. J. Physiol. 135, 93.

O'MEARA, M.P., BIRKENFELD, L.W., GOTCH, F.A. and EDELMAN, I.S. (1957).
J. Clin. Invest. 36, 784.

OSLAGE, H.J. (1965).
LandbForsch-Völkenrode 15, 107.

O'TOOLE, J.J., CECH, R.F. and PETERSON, R.E. (1967).
Metabolism 15, 1024.

PACE, N., KLINE, L., SCHACHMAN, K.H. and HARFENIST, M. (1947).
J. Biol. Chem. 168, 459.

PACE, N. and RATHBUN, E.N. (1945).
J. Biol. Chem. 158, 685.

PAINTER, E.E. (1940).
Am. J. Physiol. 129, 744.

PÁLSSON, H. and VERGÉS, J.B. (1952).
J. Agric. Sci. Camb. 42, 1.

PANARETTO, B.A. (1963).
Aust. J. Agric. Res. 14, 944.

PANARETTO, B.A. and TILL, A.R. (1963).
Aust. J. Agric. Res. 14, 926.

PAWAN, G.L.S. and CLODE, M. (1959).
Biochem. J. 74, 9.

PEARSON, A.M., PRICE, J.F., HOEFER, J.A., BRATZLER, L.J. and MAGEE, W.T. (1957).
J. Anim. Sci. 16, 481.

PEARSON, A.M., PURCHAS, R.W. and REINEKE, E.P. (1968).
in Body Composition in Animals and Man, publ. 1598.
Washington, D.C., National Academy of Sciences.

PFAU, A. (1966).
Landw. Forsch. Sonderheft No. 20, p. 139.

PRICE, J.F., PEARSON, A.M. and EMERSON, J.A. (1960).
J. Anim. Sci. 19, 786.

PRICE, J.F., PFOST, A.B., PEARSON, A.M. and HALL, C.W. (1958).
J. Anim. Sci. 17, 156.

QUENOUILLE, M.H. (1952).
Associated Measurements, p. 84. London, Butterworths Scientific Publications.

RAMSEY, C.B., WILLIAMS, J.N., HOBBS, C.S., COLE, J.W. and TEMPLE, R.S. (1965).
J. Anim. Sci. 24, 291.

REEVE, E.B. and FRANKS, J.J. (1956).
Proc. Soc. Exptl. Biol. Med. 93, 299.

REEVE, E.B., GREGERSEN, M.I., ALLEN, T.H. and SEAR, H. (1953).
Am. J. Physiol. 175, 195.

REEVE, E.B. and VEALL, N. (1949).
J. Physiol. Lond. 108, 12.

REID, J.T. (1956).
Proc. Cornell Nutrit. Conference for Feed Manufacturers.

REID, J.T., BALCH, C.C. and GLASCOCK, R.F. (1958).
Br. J. Nutr. 12, 43.

REID, J.T., BALCH, C.C., HEAD, M.J. and STROUD, J.W. (1957).
Nature, London 179, 1034.

REID, J.T., BENSADOUN, A., BULL, L.S., BURTON, J.H., GLEESON, P.A., HAN, I.K., JOO, Y.D., JOHNSON, D.E., MCMANUS, W.R., PALADINES, O.L., STROUD, J.W., TYRRELL, H.F., VAN NIEKERK, B.D.H. and WELLINGTON, G.W. (1968).
in "Body Composition in Animals and Man." Publ. No. 1598, National Academy of Sciences, Washington.

REID, J.T., BENSADOUN, A., PALADINES, O.L. and VAN NIEKERK, B.D.H. (1963).
Ann. N.Y. Acad. Sci. 110, 327.

REID, J.T., WELLINGTON, G.H. and DUNN, H.O. (1955).
J. Dairy Sci. 38, 1344.

REMENCHIK, A.P., MILLER, C.E. and KESSLER, W.V. (1968).
in "Body Composition in Animals and Man." Publ. No. 1598, National Academy of Sciences, Washington.

RITTENBERG, D. and SCHOENHEIMER, R. (1935).
J. Biol. Chem. 111, 169.

ROBERTSON, J.S. (1949).
in "Isotopic Tracers and Nuclear Radiation." 1st ed. McGraw-Hill.

ROOT, W.S., ROUGHTON, F.J.W. and GREGERSEN, M.I. (1946).
Am. J. Physiol. 146, 739.

ROSENBAUM, J.D. and LAVIETES, PhH. (1939).
J. Biol. Chem. 131, 663.

SAN PIETRO, A. and RITTENBERG, D. (1953).
J. Biol. Chem. 201, 445.

SCHEINBERG, I.H. and KOWALSKI, H.J. (1950).
J. Clin. Invest. 29, 475.

SCHLOERB, P.R., FRIIS-HANSEN, B.J., EDELMAN, I.S., SHELDON, D.B. and MOORE, F.D. (1951).
J. Lab. Clin. Med. 37, 653.

SCHLOERB, P.R., FRIIS-HANSEN, B.J., EDELMAN, I.S., SOLOMON, A.K. and MOORE, F.D. (1950).
J. Clin. Invest. 29, 1296.

SCHMIDT, J., FORSTHOFF, H. and WINZENBURGER, W. (1935).
Züchtungskunde 10, 414.

SCHÜTTERT, R., STEENBERG, K., STANDAL, N. and LEIN, H. (1968).
Acta Agric. Scand. 18, 101.

SHEPPARD, C.W. and HOUSEHOLDER, A.S. (1951).
J. Appl. Physiol. 22, 510.

SHUMWAY, R.P. (1959).
Dissertation Abstr. 20, 2465.

SIRI, W.E. (1956).
Advances in Biol. Med. Phys. 4, 239.

SIRI, W.E. (1961).
in "Techniques for Measuring Body Composition." Proc. Conf. Q.M. Res. Eng. Centre, Natick, Mass. 1959. (Ed. J. Brožek and A. Henschel), p. 108. Washington, National Academy of Sciences.

SOBERMAN, R.J. (1950).
Proc. Soc. Exptl. Biol. Med. 74, 789.

SOBERMAN, R.J., BRODIE, B.B., LEVY, B.B., AXELROD, J., HOLLANDER, V. and STEELE, J.M. (1949).
J. Biol. Chem. 179, 31.

SPRAY, G.M. and WIDDOWSON, E.M. (1951).
Brit. J. Nutr. 4, 332.

STANT, E.G. Jr., MARTIN, T.G. and KESSLER, W.V. (1969).
J. Anim. Sci. 29, 547.

STERLING, K.J. and GRAY, S. (1950).
J. Clin. Invest. 29, 1614.

STEVENS, W.H. and THURSTON, W. (1954).
Min. Rep., Atomic Energy of Canada Ltd. No. 295 (reprinted 1960).

STOUFFER, J.R., WALLENTINE, M.V. and WELLINGTON, G.H. (1959).
J. Anim. Sci. 18, 1483.

STOUFFER, J.R., WALLENTINE, M.V., WELLINGTON, G.H. and DIEKMANN, A. (1961).
J. Anim. Sci. 20, 759.

SWAN, R.C., MADISSOO, H. and PITTS, R.F. (1954).
J. Clin. Invest. 33, 1447.

TALBOT, R.B. and SWENSON, M.J. (1963).
Am. J. Vet. Res. 24, 467.

TALSO, P.J., MILLER, C.E., CARBALLO, A.J. and VASQUEZ, J. (1960).
Metabolism 9, 456.

TAYLOR, J.C. (1954).
Proc. Brit. Soc. Anim. Prod., p.3.

TULLOH, N.M. (1963).
in "Carcase Composition and Appraisal of Meat Animals."
(Ed. D.E. Tribe.) Melbourne, The Commonwealth Scientific and Industrial Research Organization.

TURNER, M.D., NEELY, W.A. and HARDY, J.D. (1960).
J. Appl. Physiol. 15, 309.

UREY, H.C. and TEAL, G.K. (1935).
Rev. med. Phys. 7, 34.

USSING, H.H. (1938).
Skand. Arch. f. Physiol. 78, 225.

VINOGRADOV, A.P. (1957).
Biokhimiya 22, 14.

VON DÜBELN, W. (1962).
quoted by Anderson, E.C. (1963). Ann. N.Y. Acad. Sci. 110, 189.

WALKER, W.G. and WILDE, W.S. (1952).
Am. J. Physiol. 170, 401.

WALLACE, G.B. and BRODIE, B.B. (1939).
J. Pharmacol. Exptl. Therap. 65, 124.

WALSER, M. (1952).
Proc. Soc. Exptl. Biol. Med. 79, 372.

WALSER, M., SELDIN, D.W. and GROLLMAN, A. (1953).
J. Clin. Invest. 32, 299.

WEDGEWOOD, R.J. (1963).
Ann. N.Y. Acad. Sci. 110, 141.

WEIL, W.B. Jr. and WALLACE, W.M. (1963).
Ann. N.Y. Acad. Sci. 110, 358.

WHITEMAN, J.V. and WHATLEY, J.A. (1953).
J. Anim. Sci. 12, 591.

WHITEMAN, J.V., WHATLEY, J.A. and HILLIER, J.C. (1953).
J. Anim. Sci. 12, 859.

WILD, J.J. (1950).
Surgery, 27, 183.

WILLMAN, J.P. and KRIDER, J.L. (1943).
J. Anim. Sci. 2, 231.

WILSON, P.N. (1960).
J. Agric. Sci. Camb. 45, 67.

WINKLER, A.W., ELKINGTON, J.R. and EISENMAN, A.J. (1943).
Am. J. Physiol. 139, 239.

WOOD, A.J. and GROVES, T.D.D. (1965).
Canad. J. Anim. Sci. 45, 8.

WOODWARD, K.T., TRUJILLO, T.T., SCHUCH, R.L. and ANDERSON, E.C. (1956).
Nature, London 178, 97.

YEH, S.-Y. and PETERSON, R.E. (1963).
J. Pharm Sci. 52, 453.

Appendix Table 1

The composition of diet N.R.S.1

Component	
Ground barley	75%
Wheatings	10%
White fish meal	6.7%
Soya-bean meal	8.3%

Supplements	(kg/ton)
Limestone	2.2
Parkhill supplement No. 2*	2.2

*Isaac Spencer & Co. (Aberdeen) Ltd., Aberdeen

2.2 kg of supplement No. 2 contains:

Vitamin A (copper stable)	2.2 mμ
D_3	0.6 mμ
B_2	2.0 g
B_{12}	5 mg
dl-calcium pantothenate	10 g
Nicotinic acid	10 g
Copper	200 ppm
Zinc	100 ppm
Magnesium	10 ppm
Manganese	30 ppm
Iron	60 ppm
Cobalt	0.9 ppm
Iodine (stabilized)	1.0 ppm

Appendix 2

METHODS OF CHEMICAL ANALYSIS OF THE CARCASS MATERIAL

Nitrogen

The determination of nitrogen was made by the macro-Kjeldahl method. Initially, about 0.5 g of the dried carcass material was weighed out onto a filter paper. This was transferred to a calibrated 800-ml Kjeldahl flask. Three Kjeltabs were added to this flask (Thomson and Capper Ltd., Liverpool; each Kjeltab contained 4.75 g K_2SO_4 and 0.25 HgO). After mixing the contents, each flask was heated on a digestion rack. When the mixture had cleared, the contents of each flask were then boiled, and digestion took place for a further two hours. The flasks were cooled, and the contents diluted to the 850-ml calibration mark with distilled water. The flask was then stoppered and vigorously shaken to ensure complete mixing.

In the second stage, nitrogen was determined as ammonia in an automated system. This was a modified Technicon unit with a recorder chart read-out. The calculations for nitrogen were made on an IBM 1130 computer (Davidson, Mathieson and Boyne, 1970). The calculated value for the percentage of nitrogen in the dried carcass material was converted to percentage protein, by multiplying by 6.25.

Ether extract

About 2 g of the dried minced carcass material were weighed into a double-walled Soxhlet thimble. This was connected up to a 150-ml flask containing 100 ml of anhydrous commercial ether. The fat was extracted by repeated refluxing for 16 hours at 75° to 80°C. After refluxing, the ether was removed by distillation into a residue bottle, and the flask and its contents were dried in an oven at 100°C for 30 minutes, or till a constant weight was achieved. The flask and contents were then cooled in a desiccator and weighed.

Estimation of total ash

Body ash was determined by one of two methods. In experiment 1, a wet ashing procedure was used. The method is described as follows.

About 0.5 g of the dried minced material was weighed into a micro-Kjeldahl flask. To this was added 0.5 ml of a concentrated sulphuric acid, 1 ml of 40% perchloric acid, and 5 ml of concentrated nitric acid. Glass beads were added to prevent bumping during the boiling process. The flasks were heated slowly at first on a small digestion rack. Boiling continued for 2 to 3 hours till the mixture became clear and a white residue could be seen. The flasks were heated to dryness, cooled and re-weighed. The weight of contents was taken as the total ash.

This was found to be a rather laborious procedure, as usually only six samples per day could be analysed. In the third experiment, the carcass samples were dry-ashed, because of the large number of samples to be analysed. Particular attention was paid to the ashing temperature, as a report in the literature (Grove, Jones and Matthews, 1961) had indicated that the maximum temperature at which animal tissue could be ashed, without loss of sodium and potassium, was 550°C in a 24-hour period.

About 2 g of the dried minced carcass material were weighed into a silica crucible and carbonized over a low gas flame. The crucible and contents were then placed in a muffle furnace at 450°C for 18 hours. When the residue became white or light grey in colour, the crucible was removed from the muffle and cooled in a desiccator and subsequently weighed.

Determination of potassium

Potassium determinations were made on the ashed carcass material in experiments 1 and 3. In experiment 1, the wet-ashed material was extracted with hot dilute hydrochloric acid and made up to 100 ml with

distilled water. Insoluble calcium salts were retained in the filter paper. The filter paper was washed with hot distilled water. The determination of potassium was made using a flame emission photometer. In experiment 1, a Carl Zeiss instrument was used (Carl Zeiss PF 5, Oberkochen) and in experiment 2, an EEL instrument (Evans Electroselenium Ltd.) was used. The full-range deflection of the former instrument for potassium was from 1 to 2 mg%, and for the latter instrument, 0 to 1 mg%. The mutual interference effects of calcium and sodium on the potassium in the ash were taken into consideration and a range of standards which were used for calibrating each instrument were constituted, containing the three elements. The ratio of these elements in the standard solution was 0.39:4.22:1 for sodium:calcium:potassium, the figures obtained from the chemical data of 90 kg pigs (Oslage, 1965).

Appendix Table 3

Individual performance data of the pigs in Experiment 3, including the values of the estimated maintenance requirements for energy, feed conversion ratio adjusted for maintenance (C.F.C.R.) and the values for the energy content of the carcasses

Pig	Age*	Days on Experiment**	Live-weight gain/day (kg)†	F.C.R.	Estimated maintenance (Mcal)‡	C.F.C.R.	Total carcass energy (Mcal)ø
1	153	78	0.77	2.77	126.6	2.04	266.5
2	166	85	0.64	3.43	136.7	2.56	286.0
3	170	92	0.69	3.26	151.5	2.45	325.4
4	178	99	0.71	3.21	163.0	2.42	349.9
5	176	99	0.56	3.50	157.1	2.54	290.0
6	181	106	0.54	3.58	174.9	2.55	315.2
7	210	133	0.43	3.81	227.3	2.47	304.5
8	232	148	0.38	3.72	243.4	2.26	259.3
9	232	155	0.42	3.61	259.7	2.25	311.6
10	223	148	0.46	3.03	260.1	1.71	236.1
11	232	155	0.37	3.71	270.2	2.09	258.9
12	272	196	0.25	4.22	351.3	1.83	199.8
13	279	203	0.29	3.96	341.6	1.97	240.5
14	290	210	0.28	4.02	369.9	1.75	203.4
15	288	217	0.29	3.77	373.1	1.69	193.7
16	185	113	0.51	3.46	178.6	2.42	268.3
17	190	120	0.53	3.41	195.8	2.35	305.1
18	175	85	0.67	2.76	134.4	1.90	219.0
19	193	92	0.58	3.03	152.7	2.00	242.0
20	198	99	0.62	2.75	168.0	1.77	229.4
21	199	106	0.58	3.23	181.3	2.21	292.6
22	212	120	0.52	2.71	212.9	1.50	235.7
23	221	127	0.52	2.91	224.1	1.71	249.7
24	223	133	0.42	3.64	217.0	2.46	273.9
Mean and S.D.	211.5±39.31	130±41.3	0.50±0.145	3.39±0.433	219.6±75.83	2.12±0.332	264.8±41.86

*Number of days from birth to slaughter

**Number of days from admission to experiment to one day prior to confinement in the metabolism cages.

†Calculated over the period from admission to the experiment to slaughter

‡Calculated using Breirem's equation (1939)

øDetermined by adiabatic bomb calorimetry on freeze-dried material from each of the carcass divisions

Appendix Table 4

The composition of the diets used in experiment 1

Diet	1	2	3
Components (%)			
Ground barley	92	80	62
Wheat offal	4	10	19
White fish meal	2	5	9
Soya-bean meal	2	5	10
	100	100	100
Supplements (kg/100 kg of main ingredients)			
Dicalcium phosphate	1.00	0.51	-
Salt	0.10	-	-
Limestone	0.67	0.40	-
Vitamin B_{12} supplement	0.01	-	-
Parkhill supplement No. 2	0.22	0.22	0.22

*Isaac Spencer & Co. (Aberdeen) Ltd., Aberdeen - Composition of the supplement given in Appendix Table 1.

Appendix Table 5

The percentage of the ^{42}K activity injected which was lost in urine, faeces and recovered from the gut contents at slaughter - Experiment 1

Pig No.	Urine	Faeces	Gut contents	Total
1	2.4	N.V.*	1.5	3.9
2	0.5	N.V.	4.2	4.7
3	1.7	N.V.	0.5	2.2
4	8.9	N.V.	0.5	9.4
5	2.1	0.2	0.8	3.1
6	2.2	0.6	1.7	4.5
7	2.6	0.5	0.5	3.6
8	5.0	0.3	0.3	5.6
9	2.6	0.1	0.2	2.9
10	5.4	N.V.	0.1	5.5
11	2.6	0.4	3.6	6.6
12	2.8	0.2	3.1	6.1
13	4.4	0.2	1.5	6.1
14	4.1	N.V.	3.6	7.7
15	1.3	0.2	0.4	1.9
16	3.2	N.V.	1.7	4.9
17	2.1	N.V.	1.1	3.2
18	3.6	1.9	2.1	7.6
19	2.2	N.V.	0.6	2.8
20	2.2	0.2	2.2	4.6
Mean	3.1%	0.2%	1.5%	4.8%
Standard Deviation	± 1.78%	± 0.42%	± 1.26%	± 1.86%

* N.V. = None voided.

Appendix Table 6

The value of exchangeable potassium calculated from the specific activity of the plasma (K_{ep}) and urine (K_{eu}), compared with the body potassium (K_c) determined chemically - Experiment 1

Pig No.	Exchangeable potassium K_{ep} (g)	Exchangeable potassium K_{eu} (g)	Chemical Potassium (K_c) (g)	$K_{ep} - K_c$ (g)	$K_{eu} - K_c$ (g)
1	-	175.1	163.5	-	+ 11.6
2	-	180.0	179.4	-	+ 0.6
3	-	158.6	155.6	-	+ 3.0
4	-	161.0	156.8	-	+ 4.2
5	-	162.2	155.0	-	+ 7.2
6	-	173.6	163.6	-	+ 10.0
7	143.2	169.1	187.3	- 44.1	- 8.2
8	152.5	153.6	165.9	- 12.3	- 12.3
9	117.0	138.1	138.1	- 21.1	0.0
10	159.5	180.7	174.8	- 15.3	+ 5.9
11	160.6	125.9	134.1	+ 26.5	- 8.2
12	133.9	164.2	173.7	- 40.2	- 9.5
13	184.1	182.1	172.6	+ 11.5	+ 9.5
14	112.7	138.3	139.8	- 27.1	- 1.6
15	153.5	181.6	176.1	- 22.6	+ 5.5
16	191.9	171.1	165.1	+ 26.8	+ 6.0
17	207.0	172.8	172.3	+ 34.7	+ 0.5
Mean for all pigs	-	163.9	163.1	-	+ 0.8
Mean for last 11 pigs	155.9	161.6	163.6	- 7.6	- 2.0

Appendix Table 7

The chemical composition of the pigs used in Experiment 1

Pig	Empty weight (kg)	Weight of dry matter (kg)	Weight of water (kg)	Weight of lipid (kg)	Fat-free weight (kg)	Fat-free dry matter (kg)	Crude protein (kg)
1	88.75	40.98	47.77	25.41	63.34	15.57	13.12
2	87.74	40.59	47.15	23.31	64.43	17.28	14.03
3	86.49	41.10	45.39	26.07	60.42	15.03	12.39
4	92.23	48.23	44.00	33.00	59.23	15.24	12.85
5	85.73	42.90	42.83	27.80	57.93	15.11	12.48
6	84.64	40.56	44.08	24.74	59.90	15.82	13.35
7	81.00	37.26	43.74	19.06	61.94	18.20	15.08
8	76.79	35.97	40.82	19.81	56.98	16.17	13.30
9	82.13	40.76	41.37	27.26	54.87	13.50	10.58
10	82.24	34.22	48.02	17.41	64.83	16.81	13.94
11	81.66	44.49	37.17	31.47	50.19	13.02	11.50
12	81.14	36.60	44.54	19.87	61.27	16.73	13.89
13	84.60	36.14	48.46	19.58	65.02	16.56	13.31
14	78.73	38.14	40.59	24.59	54.14	13.55	11.85
15	80.95	30.55	50.40	13.75	67.20	16.80	13.89
16	81.97	31.16	50.81	15.42	66.55	15.74	12.91
17	81.93	32.23	49.70	15.95	65.98	16.28	12.76
Mean	83.5	38.3	45.1	22.6	60.8	15.7	13.0
Standard Deviation	± 3.84 kg	± 4.80 kg	± 3.86 kg	± 5.60 kg	± 4.84 kg	± 1.40 kg	± 1.07 kg

Appendix Table 8

The composition of diets used in experiment 3

	Diet X (13.5% C.P. in air-dry diet)	Diet Y (22.1% C.P. in air-dry diet)
Components (%)		
Ground barley	92	62
Wheatings	4	19
White fish meal	2	9.6
Soya bean meal	2	9.4
Supplements (kg/100 kg of main ingredients)		
Limestone	0.67	-
Dicalcium phosphate	1.00	-
Salt	0.10	-
Parkhill supplement No. 2*	0.22	0.22
Vitamin B_{12} supplement	0.01	-

*Composition of Parkhill supplement No. 2 given in Appendix Table 1

Appendix Table 9(a)

The chemical composition of the 24 pigs used in Experiment 3

Pig No.	Sex	Live weight (kg)	Empty weight (kg)	Weight of dry matter (kg)	Weight of water (kg)	Weight of lipid (kg)	Weight of crude protein (kg)	Weight of ash (kg)	Fat-free weight (kg)	Fat-free dry weight (kg)
1	F	83.40	80.25	35.19	45.06	20.34	12.26	2.50	59.91	14.85
2	M	77.60	75.52	36.64	38.88	23.96	10.54	2.16	51.56	12.68
3	F	87.61	83.59	40.57	43.02	28.37	10.17	2.04	55.22	12.20
4	M	92.29	87.49	44.08	43.41	30.14	11.66	2.33	57.35	13.94
5	F	76.89	75.76	38.14	37.62	25.59	10.62	2.16	50.17	12.55
6	M	82.59	80.78	39.70	41.08	25.93	11.21	2.33	54.85	13.79
7	F	83.14	81.09	40.26	40.83	25.37	11.96	2.73	55.72	14.89
8	F	79.85	77.70	34.36	43.34	20.94	11.15	2.30	56.76	13.42
9	M	88.79	83.49	40.45	43.04	26.68	11.49	2.31	56.81	13.77
10	F	91.90	87.03	34.66	52.37	17.17	14.42	3.01	69.86	17.49
11	M	84.50	79.77	35.59	44.18	20.80	12.14	2.73	58.97	14.79
12	F	72.30	67.70	27.53	40.17	15.69	10.04	2.14	52.01	11.84
13	M	82.20	78.86	33.61	45.25	18.45	12.23	2.65	60.41	15.16
14	F	84.30	81.77	30.90	50.87	14.50	13.68	2.89	67.27	16.40
15	M	82.60	78.12	28.86	49.26	13.32	12.84	2.68	64.80	15.54
16	F	80.00	77.18	35.69	41.49	23.10	10.21	2.33	54.08	12.59
17	M	85.60	82.61	40.57	42.04	26.25	11.92	2.39	56.33	14.29
18	F	79.60	77.35	31.00	46.35	16.20	12.11	2.67	61.15	14.80
19	M	80.30	78.27	32.92	45.35	18.87	11.61	2.34	59.40	14.05
20	F	88.10	84.74	33.86	50.88	16.57	14.33	2.88	68.17	17.29
21	M	88.50	84.75	37.91	46.84	23.40	12.07	2.48	61.35	14.51
22	F	88.30	85.32	33.72	52.60	16.92	13.81	3.02	68.40	16.80
23	M	91.90	87.41	35.44	51.97	18.93	13.56	2.98	68.48	16.51
24	M	80.70	78.42	35.72	42.70	22.08	11.06	2.30	56.34	13.64
Mean		83.87	80.62	35.97	44.90	21.23	11.96	2.51	59.38	14.48
Standard Deviation		± 5.113	± 4.574	± 3.887	± 4.307	± 4.664	± 1.282	± 0.300	± 5.775	± 1.592

Appendix Table 9(b)

The physical composition of the 24 pigs used in Experiment 3

Pig No.	*Dissectible fat (kg)	*Dissectible lean (kg)	*Dissectible bone (kg)	†Dissectible fat-free weight (kg)	‡Yield of Commercial carcass (kg)
1	15.49	39.19	4.78	64.06	58.67
2	20.06	28.50	4.64	54.17	51.26
3	22.43	33.28	4.72	59.76	58.29
4	24.47	35.39	4.89	62.23	64.18
5	20.69	28.41	3.51	53.51	51.22
6	20.31	33.56	5.72	59.46	58.50
7	21.75	34.05	4.20	58.03	58.57
8	16.40	32.24	4.33	60.13	51.82
9	20.69	33.65	4.24	61.16	56.86
10	15.83	38.79	5.15	70.36	59.14
11	18.60	35.22	4.75	60.08	57.16
12	12.04	27.88	4.13	54.77	43.21
13	14.97	33.57	4.97	62.97	53.08
14	13.40	38.51	5.22	67.67	56.85
15	12.50	37.76	5.82	65.22	55.41
16	19.33	29.87	4.31	56.47	52.16
17	20.95	34.53	4.61	59.96	57.31
18	13.81	36.39	4.92	62.69	54.26
19	14.71	36.79	4.49	62.60	54.94
20	15.46	38.55	4.37	68.52	59.33
21	18.96	36.07	4.45	64.64	58.52
22	14.97	40.23	4.65	69.49	59.43
23	17.10	37.20	4.67	69.36	58.48
24	19.31	32.11	4.00	57.92	54.14
Mean	17.71	34.65	4.65	61.84	55.95
Standard Deviation	± 3.371 kg	± 3.545 kg	± 0.514 kg	± 4.835 kg	± 4.167 kg

*Calculated as Weight of dissectible component in Physically Dissected Side (PDS) x $\frac{\text{Weight of PDS + Jointed Side}}{\text{Weight of PDS}}$

†Calculated as Empty body weight - wt of dissectible fat

‡Calculated as Empty body weight - (wt of head + internal organs + alimentary tract + chine)

Appendix Table 10

The percentage of the total ^{42}K activity which was lost in the urine, faeces and recovered from the gut contents at slaughter - Experiment 3

Pig	Urine	Faeces	Gut contents	Total
1	2.13	NV†	0.73	2.86
2	4.58	0.32	0.64	5.54
3	2.57	NV	1.23	3.80
4	3.60	0.09	1.34	5.03
5	3.27	NV	0.76	4.03
6	3.54	0.17	0.99	4.70
7	3.21	0.19	0.98	4.38
8	1.43	0.25	1.22	2.90
9	4.63	0.24	1.49	6.36
10	1.32	0.04	1.53	2.89
11	3.44	0.41	1.08	4.93
12	0.59	0.68	1.96	3.23
13	0.66	0.49	1.23	2.38
14	1.26	0.33	0.49	2.08
15	2.44	0.22	0.81	3.47
16	0.23	0.31	0.94	1.48
17	4.32	NV	1.05	5.37
*19	2.51	NV	0.97	3.48
20	0.81	NV	1.28	2.09
*22	2.57	0.25	0.79	3.61
23	3.07	0.22	2.79	6.08
24	0.90	0.07	0.81	1.78
Mean and Standard Deviation	‡2.41±1.352	‡0.19±0.183	‡1.14±0.497	‡3.75±1.408

*^{42}K determinations were not made on Pigs 18 and 21

†NV = none voided

‡Mean of 22 values

Appendix Table 11

The values of exchangeable potassium calculated from the specific activity of the plasma (K_{ep}) and urine (K_{eu}) compared with the body potassium (K_c) determined chemically - Experiment 3

Pig	Exchangeable potassium K_{ep} (g)	Exchangeable potassium K_{eu} (g)	Chemical potassium (K_c) (g)	$K_{ep} - K_c$ (g)	$K_{eu} - K_c$ (g)
1	154.4	157.4	165.2	-10.8	- 7.8
2	139.9	140.2	141.3	- 1.4	- 1.1
3	145.9	148.0	140.9	5.0	7.1
4	149.5	147.0	156.2	- 6.7	- 9.2
5	135.8	136.5	138.2	- 2.4	- 1.7
6	140.2	139.4	148.3	- 8.1	- 8.9
7	146.8	147.4	159.1	-12.3	-11.7
8	145.2	150.9	150.3	- 5.1	0.6
9	151.2	155.1	153.7	- 2.5	1.4
10	179.2	176.0	189.0	- 9.8	-13.0
11	149.7	155.1	163.3	-13.6	- 8.2
12	141.0	142.1	137.7	3.3	4.4
13	153.7	158.2	163.1	- 9.4	- 4.9
14	179.7	181.8	181.9	- 2.2	- 0.1
15	168.0	163.0	171.7	- 3.7	- 8.7
16	139.3	144.3	141.5	- 2.2	2.8
17	141.4	141.6	151.4	-10.0	- 9.8
*19	153.7	159.0	156.2	- 2.5	2.8
20	175.6	177.7	184.2	- 8.6	- 6.5
*22	174.4	179.3	184.8	-10.4	- 5.5
23	177.1	176.0	181.8	- 4.7	- 5.8
24	152.1	150.0	149.9	- 2.2	0.1
Mean	154.2±14.50	155.7±14.25	159.5±16.44	- 5.5	- 3.8

*^{42}K determinations were not made on Pigs 18 and 21

Appendix Table 12

Individual values for the estimates of empty body water (EBW), total body water (TBW) and deuterium oxide space (D_2O) for 24 pigs - Experiment 3

Pig	EBW (kg)	TBW (kg)	D_2O (kg)	D_2O/EBW (%)	D_2O/TBW (%)
1	45.06	47.71	47.51	105.4	99.6
2	38.88	40.64	41.10	108.3	103.6
3	43.02	46.56	47.53	110.5	102.1
4	43.41	47.49	48.80	112.4	102.8
5	37.62	38.54	40.88	108.7	106.1
6	41.08	42.57	44.71	108.8	105.0
7	40.83	42.51	44.12	108.1	103.8
8	43.34	45.22	46.01	106.2	101.8
9	43.04	47.67	49.53	115.1	103.9
10	52.37	56.79	58.23	111.2	102.5
11	44.18	48.10	48.28	109.3	100.4
12	40.17	44.21	46.33	115.3	104.8
13	45.25	48.02	48.73	107.7	101.5
14	50.87	52.99	54.96	108.0	103.7
15	49.26	53.68	54.15	109.9	100.9
16	41.49	43.81	43.10	103.9	98.4
17	42.04	44.41	44.79	106.5	100.9
18	46.35	48.08	49.31	106.4	102.6
19	45.35	47.02	47.79	105.4	101.6
20	50.88	53.58	53.00	104.2	98.9
21	46.84	49.99	50.11	107.0	100.2
22	51.60	54.10	56.16	108.8	103.8
23	51.97	55.89	56.96	110.4	101.9
24	42.70	44.59	45.15	107.1	101.3
Mean	44.90±4.307	47.65±4.797	48.67±4.766	108.5	102.2

Appendix Table 13

Individual values for the estimates of blood volume (litres), plasma volume (litres), red cell volume (litres) and haematocrit (%) on 24 animals - Experiment 3

Pig	Blood volume (l)	Plasma volume (l)	*Red cell volume (l)	‡Haematocrit (%)
1	7.79	4.96	2.83	36.3
2	6.07	4.06	2.01	33.1
3	7.15	4.72	2.43	34.0
4	7.63	5.10	2.53	37.7
5	7.23	4.55	2.68	37.1
6	6.40	4.38	2.02	31.5
7	6.92	4.80	2.12	30.7
8	7.46	4.85	2.61	34.9
9	7.40	5.05	2.35	31.8
10	8.31	5.11	3.20	38.5
11	7.82	5.23	2.59	33.1
12	5.93	3.86	2.07	35.0
13	7.13	4.89	2.24	31.5
14	8.47	5.44	3.03	35.8
15	7.52	5.04	2.48	33.0
16	6.72	4.60	2.12	31.5
17	7.44	4.92	2.52	33.9
18	7.48	4.89	2.59	34.7
19	7.70	5.12	2.58	33.5
20	8.38	5.28	3.10	37.0
21	7.92	5.17	2.75	34.8
22	8.12	5.52	2.60	32.0
23	8.25	5.36	2.89	35.0
24	6.54	4.41	2.13	32.6
Mean	7.40±0.706	4.89±0.414	2.51±3.463	34.1±2.121

*Calculated from: Blood volume - Plasma volume

‡Mean value of 30 and 60 minute haematocrit values

Appendix Table 14

Individual values of external dimensions† of 24 pigs - Experiment 3

Pig	Neck circumference (cm)	Heart girth (cm)	Height at shoulder (cm)	Length of body (cm)	Length of forearm (cm)	Length of hind leg (cm)	Jaw length (cm)	Jaw width (cm)
1	73.5	101.0	52.5	77.0	35.0	23.5	**19.5**	18.5
2	74.0	107.0	57.0	86.0	32.0	25.0	**21.5**	18.5
3	74.5	102.5	59.0	86.5	32.0	21.5	20.5	17.0
4	85.0	106.0	64.0	92.0	32.5	26.0	19.5	18.5
5	71.5	99.5	56.5	84.5	30.0	24.0	18.0	16.5
6	76.5	102.0	57.5	93.0	36.5	28.0	22.0	16.5
7	77.5	107.0	56.0	91.5	34.0	26.5	24.0	17.5
8	73.0	106.0	61.0	92.0	36.0	26.0	19.0	18.5
9	77.0	104.0	64.0	93.0	38.0	28.5	21.5	17.0
10	71.0	104.0	64.0	100.0	37.5	27.0	23.5	17.0
11	74.5	102.0	60.0	89.0	34.5	26.5	22.5	18.5
12	MV*	MV*	62.5	87.5	33.5	25.5	21.5	17.5
13	67.5	96.5	61.0	89.0	36.5	28.5	22.5	17.0
14	68.0	97.5	60.0	92.0	36.0	27.5	23.5	17.0
15	69.0	**101.5**	61.0	94.0	37.0	28.0	27.0	16.5
16	71.5	94.0	58.5	99.0	26.0	24.5	22.5	19.5
17	73.5	98.0	62.0	96.5	36.0	27.5	19.0	16.0
18	75.0	100.5	59.0	88.0	33.0	27.0	20.0	18.5
19	70.5	98.5	58.0	89.0	36.5	27.5	23.0	16.0
20	69.5	104.0	60.0	93.5	37.0	29.0	25.0	17.5
21	76.5	109.5	61.0	94.0	36.0	27.0	22.5	15.5
22	75.0	105.5	63.0	95.5	35.0	28.5	23.0	16.0
23	72.0	99.0	66.5	97.5	38.5	29.0	24.0	18.0
24	70.0	97.0	64.0	89.5	37.5	29.0	21.0	17.0
Mean	‡73.3±3.79	‡102.0±4.22	60.2±3.13	91.2±5.07	35.1±2.11	26.8±1.84	22.1±2.03	17.3±1.06

*MV - missing value

†Descriptions of these measurements are given in Chapter 6

‡Mean of 23 values

Appendix Table 15

Individual values of backfat measurements made on the live animal by ultrasonics, and on the chilled carcass, by calipers - Experiment 3

Pig	Maximum shoulder fat (mm)		Minimum midback (mm)		Minimum loin fat (mm)		C (mm)		K (mm)	
	Ultrasonics*	Calipers‡	Ultrasonics*	Calipers‡	Ultrasonics*	Calipers‡	Ultrasonics*	Calipers†‡	Ultrasonics*	Calipers‡ø
1	44.33	49.00	23.67	21.00	25.67	24.00	21.33	17.00	23.33	18.00
2	48.33	51.00	20.00	23.00	26.00	22.00	17.67	24.00	22.67	26.00
3	52.50[a]	54.00	23.75[a]	23.00	26.50[a]	35.00	22.75[a]	26.00	23.25[a]	28.00
4	57.75[a]	60.00	23.75[a]	26.00	29.50[a]	35.50	20.00[a]	25.00	22.75[a]	27.00
5	56.33	58.00	31.67	29.00	33.17	31.50	27.67	30.00	31.50	32.00
6	42.83	57.00	21.50	22.00	21.83	22.00	18.67	20.50	21.33	23.50
7	46.67	42.00	22.33	22.00	24.00	23.00	21.50	22.00	22.17	24.00
8	37.25[a]	34.00	14.25[a]	17.50	18.75[a]	16.00	12.50[a]	21.00	14.25[a]	22.50
9	44.67	43.50	16.33	20.50	22.17	20.00	12.17	22.50	13.50	29.00
10	36.75[a]	41.00	13.75[a]	11.00	18.00[a]	12.00	14.25[a]	12.00	17.25[a]	14.00
11	41.83	45.00	19.83	20.50	21.67	20.50	17.67	19.00	19.33	22.00
12	37.50	37.50	13.50	11.00	16.00	13.50	13.00	10.50	14.00	12.00
13	37.00	37.00	10.33	13.50	14.67	13.50	14.67	14.50	16.00	15.50
14	36.00	36.50	12.00	14.00	14.50	9.50	11.00	8.50	13.00	8.00
15	38.00	41.50	11.83	10.50	14.50	11.00	11.83	9.50	13.00	10.00
16	44.00	45.50	17.67	15.00	20.33	25.50	12.00	16.00	14.50	17.00
17	43.17	44.00	24.00	29.00	28.00[a]	33.00	24.67[a]	25.50	25.00	30.00
18	41.50[a]	40.50	13.50[a]	15.00	17.25[a]	13.00	13.25[a]	15.50	16.75[a]	16.50
19	37.83	35.00	14.50	18.50	16.33	17.00	12.67	14.50	14.83	16.50
20	45.33	46.00	17.83	19.00	18.67	24.00	16.17	15.50	17.16	17.50
21	52.17	52.00	21.17	23.00	22.67	21.00	17.83	19.50	19.17	21.00
22	42.17	38.00	17.17	15.50	16.83	17.00	13.67	15.00	15.50	17.00
23	42.33	45.00	15.83	18.50	19.17	20.50	13.83	16.50	15.83	17.00
24	41.83	42.50	17.67	22.00	18.83	25.00	10.83	11.00	12.67	14.10
Mean and S.D.	43.69±6.121	44.81±7.371	18.20±5.095	19.16±5.251	21.04±5.070	21.04±7.427	16.31±4.661	17.95±5.751	18.28±4.80	19.92±6.508

*Mean value of three operators except where indicated (a)
(a) Mean value of two operators
‡Mean value of four readings taken on the chilled hanging carcass
†Measured $4\frac{1}{2}$ cm from the midline - the mean value of four readings
øMeasured 8 cm from the midline - the mean value of four readings

Appendix Table 16

The individual scores of each of the judges on the visual appraisal panel - Experiment 3

Pig	Judge A	B	C	D	E	Mean
1	3	2	3	3	3	2.8
2	3	2	4	3	3	3.0
3	3	2	3	MV	2	2.5
4	2	MV	2	3	2	2.3
5	2	MV	3	3	3	2.8
6	3	3	3	3	3	3.0
7	2	3	2	3	3	2.6
8	MV	4	MV	4	2	3.3
9	3	2	MV	3	3	2.8
10	3	3	4	MV	3	3.3
11	3	4	4	4	MV	3.8
12	4	4	3	MV	4	3.8
13	3	4	4	5	3	3.8
14	4	3	3	5	4	3.8
15	4	5	5	5	4	4.6
16	2	3	3	3	3	2.8
17	2	3	3	2	2	2.4
18†						
19	3	4	4	3	3	3.4
20	3	3	4	2	3	3.0
21	3	3	3	3	2	2.8
22	3	4	4	4	3	3.6
23	3	4	4	5	4	4.0
24	3	4	3	3	MV	3.3
Mean*	2.9	3.3	3.4	3.5	2.8	3.2 ± 0.59

*This value is not the same as that given in the text because the latter was calculated from the scores of all the pigs.

MV = missing value

†No scores were recorded for pig 18 because of low attendance of the panel judges

Appendix Table 17

Individual values for specific gravity, muscle/fat ratio and muscle/bone ratio of the physically dissected side for 24 pigs - Experiment 3

Pig	Specific gravity of PDS*	Muscle/bone in PDS†	Muscle/fat in PDS†
1	1.0469	8.21	2.53
2	1.0365	6.26	1.45
3	1.0348	7.06	1.48
4	1.0274	7.24	1.45
5	1.0330	8.09	1.37
6	1.0336	5.87	1.61
7	1.0454	8.12	1.57
8	1.0476	7.45	1.97
9	1.0425	7.94	1.63
10	1.0616	7.53	2.45
11	1.0483	7.42	1.89
12	1.0531	6.76	2.32
13	1.0561	6.76	2.19
14	1.0614	7.38	2.87
15	1.0626	6.49	3.02
16	1.0397	6.94	1.55
17	1.0351	7.50	1.65
18	1.0582	7.39	2.64
19	1.0543	8.19	2.50
20	1.0585	8.81	2.34
21	1.0451	8.11	1.90
22	1.0575	8.65	2.69
23	1.0539	7.97	2.18
24	1.0409	8.02	1.66
Mean	1.0473±0.01049	7.51±0.744	2.04±0.506

*Water temperature = 12.5°C

†Dissectible components of the physically dissected side

Appendix Table 18a

The correlation* between each of the measurements and each of the body components in Experiment 3

	Water (kg)	Lipid (kg)	Protein (kg)	Ash (kg)	FFBM (kg)	FFDM (kg)	Diss. FFBM (kg)	Diss. Bone (kg)	Diss. Fat (kg)	Diss. Lean (kg)	Carcass Yield (kg)	Potassium (g)	Energy (Kcal)
Live weight at slaughter (kg)	0.621	-0.005	0.591	0.512	0.621	0.572	0.706	0.274	0.089	0.630	0.843	0.615	0.086
Empty body weight (kg)	0.611	0.015	0.607	0.521	0.618	0.589	0.707	0.227	0.111	0.635	0.866	0.622	0.114
Specific gravity of the physically dissected side of the carcass	0.837	-0.944	0.793	0.799	0.846	0.808	0.765	0.331	-0.899	0.692	0.028	0.809	-0.919
Mean visual assessment score	0.597	-0.835	0.479	0.556	0.583	0.500	0.487	0.447	-0.809	0.382	-0.213	0.507	-0.837
Feed conversion ratio (unadjusted)	-0.394	0.083	-0.350	-0.224	-0.383	-0.323	-0.443	0.099	0.065	-0.462	-0.448	-0.352	0.013
Total age (days)	0.491	-0.633	0.449	0.508	0.495	0.467	0.418	0.358	-0.597	0.296	-0.134	0.469	-0.649
Blood volume (litres)	0.808	-0.530	0.808	0.710	0.812	0.763	0.828	0.112	-0.478	0.797	0.492	0.814	-0.488
Plasma volume (litres)	0.806	-0.524	0.779	0.746	0.809	0.754	0.832	0.198	-0.480	0.833	0.541	0.807	-0.479
Red cell volume (litres)	0.682	-0.452	0.712	0.561	0.688	0.652	0.694	0.005	-0.401	0.633	0.364	0.694	-0.420
Deuterium oxide space (kg)	0.970	-0.669	0.877	0.814	0.961	0.862	0.963	0.468	-0.589	0.837	0.503	0.905	-0.599
Exchangeable potassium (g)	0.973	-0.771	0.921	0.857	0.977	0.910	0.935	0.361	-0.664	0.826	0.416	0.954	-0.706
Neck circumference (cm)	-0.375	0.751	-0.302	-0.296	-0.365	-0.310	-0.249	-0.073	0.765	-0.138	0.536	-0.299	0.806
Heart girth (cm)	-0.031	0.181	0.073	0.007	-0.009	0.051	0.023	-0.007	0.213	0.052	0.260	0.039	0.243
Shoulder height (cm)	0.520	-0.118	0.424	0.392	0.501	0.411	0.510	0.138	-0.014	0.279	0.337	0.453	-0.091
Length of body (cm)	0.437	-0.189	0.398	0.462	0.440	0.416	0.395	0.215	-0.060	0.202	0.213	0.420	-0.187
Length of forearm (cm)	0.644	-0.540	0.598	0.561	0.652	0.626	0.662	0.396	-0.545	0.570	0.197	0.624	-0.488
Length of hind leg (cm)	0.490	-0.476	0.557	0.565	0.527	0.588	0.475	0.198	-0.410	0.358	0.092	0.548	-0.439
Length of jaw (cm)	0.617	-0.719	0.538	0.616	0.621	0.585	0.499	0.390	-0.594	0.429	0.034	0.552	-0.695
Width of jaw (cm)	-0.174	0.181	-0.206	-0.070	-0.177	-0.174	-0.235	-0.155	0.293	-0.236	0.024	-0.176	0.178
Maximum shoulder fat depth by ultrasonics (mm)	-0.536	0.751	-0.464	-0.508	-0.538	-0.502	-0.490	-0.403	0.797	-0.428	0.203	-0.507	0.742
Minimum midback fat depth by ultrasonics (mm)	-0.638	0.725	0.475	-0.505	-0.616	-0.507	-0.572	-0.433	0.728	-0.406	0.116	-0.520	0.712
Minimum loin fat depth by ultrasonics (mm)	-0.681	0.816	-0.528	-0.582	-0.665	-0.572	-0.599	-0.419	0.803	-0.489	0.090	-0.566	0.797
C fat depth measured by ultrasonics (mm)	-0.522	0.606	-0.311	-0.358	-0.484	-0.340	-0.427	-0.239	0.589	-0.273	0.156	-0.360	0.601
K fat depth measured by ultrasonics (mm)	-0.561	0.604	-0.348	-0.395	-0.524	-0.381	-0.476	-0.247	0.588	-0.351	0.080	-0.403	0.596
Depth of shoulder fat on the chilled carcass (mm)	-0.479	0.674	-0.410	-0.470	-0.478	-0.439	-0.418	-0.032	0.706	-0.365	0.255	-0.456	0.688
Depth of midback fat on the chilled carcass (mm)	-0.690	0.799	-0.525	-0.598	-0.669	-0.560	-0.612	-0.492	0.785	-0.470	0.073	-0.567	0.787
Depth of loin fat on the chilled carcass (mm)	-0.576	0.821	-0.540	-0.589	-0.585	-0.565	-0.520	-0.440	0.840	-0.418	0.204	-0.555	0.807
C fat depth on the chilled carcass (mm)	-0.714	0.849	-0.592	-0.622	-0.706	-0.630	-0.626	-0.430	0.803	-0.579	-0.019	-0.634	0.820
K fat depth on the chilled carcass (mm)	-0.715	0.870	-0.580	-0.625	-0.703	-0.617	-0.619	-0.444	0.823	-0.562	0.004	-0.622	0.845
Feed conversion ratio adjusted for maintenance	-0.948	0.859	-0.881	-0.845	-0.947	-0.873	-0.916	-0.381	0.814	-0.837	-0.278	-0.906	-0.812

*Correlations based on the data obtained from 21 animals (19 degrees of freedom)

$P < 0.05$ when $\pm r > 0.433$, $P < 0.01$ when $\pm r > 0.549$ and $P < 0.001$ when $\pm r > 0.665$

Appendix Table 18b

The correlations* between each of the measurements which were made in Experiment 3

	Live weight at slaughter (kg)	Empty body weight (kg)	Specific gravity of the physically dissected side of the carcass	Mean visual assessment score	Feed conversion ratio (unadjusted)	Total age (days)	Blood volume (litres)	Plasma volume (litres)	Red cell volume (litres)	Deuterium oxide space (kg)	Exchangeable potassium (g)	Neck circumference (cm)	Heart girth (cm)	Shoulder height (cm)	Length of body (cm)	Length of forearm (cm)	Length of hind leg (cm)	Length of jaw (cm)	Width of jaw (cm)	Maximum shoulder fat depth by ultrasonics (mm)	Minimum midback fat depth by ultrasonics (mm)	Minimum loin fat depth by ultrasonics (mm)	C fat depth measured by ultrasonics (mm)	K fat depth measured by ultrasonics (mm)	Depth of shoulder fat on the chilled carcass (mm)	Depth of midback fat on the chilled carcass (mm)	Depth of loin fat on the chilled carcass (mm)	C fat depth on the chilled carcass (mm)	K fat depth on the chilled carcass (mm)	Feed conversion ratio adjusted for maintenance
Live weight at slaughter (kg)	1.000																													
Empty body weight (kg)	0.979	1.000																												
Specific gravity of the physically dissected side of the carcass	0.193	0.165	1.000																											
Mean visual assessment score	-0.053	-0.118	0.770	1.000																										
Feed conversion ratio (unadjusted)	-0.456	-0.504	-0.094	0.186	1.000																									
Total age (days)	0.065	-0.005	0.680	0.758	0.566	1.000																								
Blood volume (litres)	0.624	0.628	0.626	0.289	-0.430	0.293	1.000																							
Plasma volume (litres)	0.645	0.630	0.650	0.387	-0.318	0.428	0.928	1.000																						
Red cell volume (litres)	0.504	0.528	0.504	0.141	-0.480	0.105	0.919	0.705	1.000																					
Deuterium oxide space (kg)	0.720	0.691	0.769	0.567	-0.345	0.512	0.789	0.806	0.648	1.000																				
Exchangeable potassium (g)	0.587	0.537	0.835	0.580	-0.341	0.512	0.784	0.761	0.684	0.953	1.000																			
Neck circumference (cm)	0.346	0.358	-0.661	-0.600	-0.109	-0.467	-0.211	-0.112	-0.283	0.241	-0.377	1.000																		
Heart girth (cm)	0.197	0.211	-0.078	-0.190	-0.132	-0.159	0.008	-0.007	0.022	0.051	-0.003	0.526	1.000																	
Shoulder height (cm)	0.695	0.649	0.218	0.265	-0.097	0.413	0.382	0.466	0.233	0.619	0.493	0.116	-0.022	1.000																
Length of body (cm)	0.462	0.463	0.267	0.185	-0.025	0.329	0.225	0.316	0.094	0.459	0.388	-0.007	-0.098	0.708	1.000															
Length of forearm (cm)	0.395	0.361	0.635	0.494	-0.079	0.519	0.402	0.479	0.258	0.619	0.592	-0.345	-0.175	0.474	0.514	1.000														
Length of hind leg (cm)	0.234	0.242	0.511	0.499	-0.072	0.580	0.265	0.409	0.072	0.463	0.485	-0.200	-0.088	0.573	0.620	0.777	1.000													
Length of jaw (cm)	0.124	0.087	0.760	0.712	-0.087	0.626	0.208	0.332	0.044	0.567	0.632	-0.413	0.023	0.260	0.463	0.600	0.595	1.000												
Width of jaw (cm)	0.006	-0.055	-0.251	-0.173	-0.038	-0.251	-0.221	-0.242	-0.164	-0.197	-0.135	0.193	-0.146	-0.091	-0.113	-0.206	-0.236	-0.031	1.000											
Maximum shoulder fat depth by ultrasonics (mm)	0.057	0.084	-0.763	-0.693	-0.202	-0.672	-0.276	-0.336	-0.169	-0.456	-0.481	0.630	0.225	-0.158	-0.314	-0.761	-0.587	-0.610	0.285	1.000										
Minimum midback fat depth by ultrasonics (mm)	-0.131	-0.073	-0.778	-0.725	-0.181	-0.766	-0.279	-0.398	-0.111	-0.606	-0.602	0.494	0.105	-0.409	-0.416	-0.721	-0.637	-0.744	0.139	0.837	1.000									
Minimum loin fat depth by ultrasonics (mm)	-0.066	-0.037	-0.840	-0.779	-0.123	-0.738	-0.309	-0.431	-0.133	-0.607	-0.646	0.578	0.188	-0.293	-0.388	-0.720	-0.656	-0.821	0.182	0.870	0.945	1.000								
C fat depth measured by ultrasonics (mm)	-0.066	-0.012	-0.607	-0.625	-0.124	-0.615	-0.170	-0.288	-0.018	-0.488	-0.509	0.349	0.116	-0.410	-0.419	-0.641	-0.618	-0.700	-0.070	0.702	0.893	0.866	1.000							
K fat depth measured by ultrasonics (mm)	-0.135	-0.078	-0.640	-0.615	-0.139	-0.666	-0.224	-0.374	-0.031	-0.522	-0.533	0.356	0.134	-0.434	-0.442	-0.707	-0.645	-0.733	-0.008	0.735	0.905	0.894	0.976	1.000						
Depth of shoulder fat on the chilled carcass (mm)	0.070	0.083	-0.751	-0.561	-0.172	-0.656	-0.339	-0.438	-0.181	-0.395	-0.448	0.545	0.091	-0.186	-0.268	-0.582	-0.531	-0.552	0.323	0.682	0.789	0.791	0.670	0.728	1.000					
Depth of midback fat on the chilled carcass (mm)	-0.128	-0.065	-0.836	-0.724	-0.067	-0.669	-0.330	-0.404	-0.201	-0.636	-0.650	0.540	0.105	-0.228	-0.383	-0.576	-0.411	-0.762	0.060	0.750	0.976	0.883	0.772	0.769	0.649	1.000				
Depth of loin fat on the chilled carcass	0.059	0.096	-0.844	-0.799	-0.235	-0.749	-0.300	-0.358	-0.191	-0.554	-0.588	0.561	0.004	-0.130	-0.223	-0.607	-0.539	-0.694	0.259	0.852	0.850	0.854	0.724	0.695	0.743	0.853	1.000			
C fat depth on the chilled carcass	-0.088	-0.060	-0.809	-0.720	-0.019	-0.636	-0.355	-0.410	-0.240	-0.624	-0.716	0.588	0.331	-0.217	-0.300	-0.642	-0.529	-0.758	-0.046	0.781	0.804	0.891	0.790	0.797	0.643	0.830	0.759	1.000		
K fat depth on the chilled carcass	-0.047	-0.029	-0.809	-0.738	-0.012	-0.613	-0.365	-0.402	-0.269	-0.612	-0.716	0.615	0.332	-0.146	-0.246	-0.548	-0.439	-0.742	-0.073	0.733	0.771	0.862	0.736	0.733	0.602	0.837	0.745	0.980	1.000	
Feed conversion ratio adjusted for maintenance	-0.456	-0.431	-0.905	-0.699	0.352	-0.543	-0.794	-0.825	-0.637	-0.909	-0.914	0.476	0.068	-0.409	-0.324	-0.605	-0.493	-0.596	0.258	0.588	0.649	0.700	0.499	0.534	0.569	0.717	0.664	0.702	0.705	1.000

*Correlations based on the data obtained from 21 animals (19 degrees of freedom)

$P < 0.05$ when $r > 0.433$, $P < 0.01$ when $r > 0.549$ and $P < 0.001$ when $r > 0.666$